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VOLUME IV 1925

MEDICINE

Analytical Reviews
of
General Medicine
Neurology and Pediatrics

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THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MD.
1925

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LEAD POISONING

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SECTION XX BY ALICE HAMILTON

From the Department of Physiology, Harvard School of Public Health, and the Medical Clinic Massachusetts General Hospital

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INTRODUCTION[1]

Lead intoxication is not only the most common poisoning in industry, but also is derived from such diverse non-industrial sources as water supplies, cosmetics and drugs, and home-made beverages. It is a preventable disease which may be considered from at least three points of view. In industry the necessity of preventive measures has long been realized and much has been written in regard to methods to reduce the lead hazard by improving conditions of work. The legal aspect of the disease has been well summarized recently by Miss Hutton (222) and by Cantineau (65) and no further review is necessary here. The biological problems involved in lead poisoning are of much scientific and practical interest. This monograph deals with this aspect of the disease and discusses in some detail recent work which was undertaken to help explain the reactions produced by lead in the body.

[1] The funds for this work were given to the Harvard Medical School by the National Lead Institute. We are indebted to Drs. Albert Key and Hermann Blumgart, and also to Drs. A. V. Bock, J. W. S. Brady, E. J. Cohn, Wm. A. Hinton, Chester Jones, and Misses J. Hendry and A. Hopkins for assistance in various portions of the experimental work, to Misses Charlotte Shaw, Mary Morrison, Agnes Peltz, Helen Tracy, and Mrs. Marion Frankfurter for technical aid and to Miss Martha Taylor for invaluable aid in preparing this manuscript. To Dr. C. K. Drinker, in whose laboratory our work was done, to Dean David Edsall, and to Prof. A. B. Lamb and Dr. Alice Hamilton, we are grateful for valuable aid and counsel.

No attempt has been made to cover fully the literature of the subject. Three thousand references were collected and published by Else Blänsdorf in 1922 (38) and our files contain more than 2200. Only those references which bear directly upon the discussion are included here, although we have tried to summarize and include all the pertinent literature in order to obtain a fair perspective of present knowledge.

The problems have been approached from their physiological and chemical aspects in order to obtain explanation, wherever possible, of the mechanisms involved in the production of the various conditions associated with the disease. The first portion of the monograph is therefore composed of a consideration of the chemical problems associated with lead in the organism. It includes methods of analysis and a description of the reactions of lead in the body during absorption, storage, and excretion. A summary of the literature of the gross and microscopic pathology follows and leads to a discussion of the various toxic manifestations of plumbism in the light of the data bearing on the physiology of their production. Finally, the clinical picture is given and an outline of the methods of treatment. At the end is a summary of the present industrial situation very kindly contributed by Dr. Alice Hamilton.

I. HISTORY

Even in ancient times the disease which is now known as lead poisoning was recognized. As both Tanquerel des Planches (453) and Alderson (5) have very fully outlined the ancient history of lead poisoning, only a very brief statement will be included here. The many symptoms of plumbism were noted long before they were ascribed to the action of lead. Gradually, however, physicians came to appreciate the cause of these disturbances and the syndrome was designated "saturnism," for lead was "called saturn by the alchemists because it absorbs and devours, so to speak, all other imperfect metals in its scorification" (147). For many years only clinical descriptions appeared in the literature, and it was not until the early part of the nineteenth century that experimental investigation of the action of lead in the body was begun. This has continued until the present day, and recently has been supplemented by attempts to improve

industrial conditions. Consequently the incidence of plumbism has been markedly reduced.

Hippocrates (370 B.C.) was probably the first of the ancients to recognize lead as the cause of symptoms, at least he describes a severe attack of colic in a man who extracted metals. The relationship of constipation, abdominal pain, and pallor to the action of lead on the body was also observed by Nicander in the second century B.C. This author also mentioned lead palsy for the first time. But probably not until the first century A.D. was the true significance of lead appreciated. Dioscorides is quoted by Alderson as saying that "the drinking of litharge (red lead) causes oppression to the stomach, belly, and intestines, with intense wringing pains; sometimes it even wounds the intestines by its severe pressure; it suppresses the urine, while the body swells and acquires an unsightly leaden hue." He also speaks of paralysis and delirium as consequences of ingestion of lead. Many years later, about 1000 A.D., Avicenna recommended the use of violent purgatives in doses graded according to the severity of the attack for a special form of colic which must have been caused by lead. Crato who lived about 1600, definitely attributed the colic which was common in Moravia and was known to end in paralysis to the use of "falsified" wine; but it was not until 1656 that paralysis was definitely recognized as an effect of lead. Stockhusen (444) then published a remarkable treatise in which he recognized that lead poisoning followed absorption of lead dust. He also tried to determine which muscles of the arm are commonly paralyzed.

In 1616 Citois (81) a physician of Poitiers, also mentioned bad wine which had been treated with lead as the cause of colic, and described the disease (*Colica Pictonum*) excellently although he attributed the disturbance chiefly to the acid of the wine. Tronchin (467) pointed out in 1757 the relationship between this type of colic and the disease common among lead workers. In 1745 Huxham (223) described a disease appearing in Devonshire, England, the symptoms of which were identical with true lead colic. This was later (1767) traced by Sir George Baker to the lead employed in the manufacture and storage of cider. In Spain a similar type of colic of similar etiology was described in 1796 by Luzuriaga (271). Shortly after this, in the beginning of the nineteenth century, several brilliant

clinical descriptions appeared: Grisolle (178) described colic, lead line, and particularly encephalopathy very accurately; and three years later the epoch-making book by Tanquerel des Planches was published. In this the clinical aspect of the disease is quite perfectly and completely outlined and most of the acute signs of lead poisoning are mentioned. In spite of the fact that they laid emphasis on the lead line, this sign has come to be known as the Burtonian line because of a description published by Burton (59) in 1840. Since Tanquerel's time little has been added to our clinical knowledge of lead poisoning except by Déjérine-Klumpke (92) who wrote an excellent treatise on the lead palsies. Several good discussions of the early differential diagnosis of plumbism have appeared recently.

Experimental study of the disease was apparently started in 1814 by Orfila (344) who administered lead intravenously and by mouth. He observed that its toxic action was greater after oral than after intravenous administration, and also that it was not a very poisonous metal. Grisolle and Tanquerel also did some experimental work, though their contributions in this line were not striking. They studied the toxicity, paths of entry and distribution of lead in the organism as well as the clinical picture of poisoning in animals. Since their time many investigations have been carried out. As these are reported in the chapters which follow, they need not be re-summarized here; but it should be stated that they have dealt with the modes of absorption and excretion of lead, and to a slight extent with an explanation of the mechanism of its action in the body. Although some work has been centered upon the problem of palsy, most interest has been given to the obvious changes caused by lead in the blood. Between 1865 and the beginning of the present century attention was focused upon the pathology of lead poisoning, but this produced very little new fundamental knowledge. More recently, modern methods of histological staining have enabled some slight advance to be made in studies of the pathology of encephalopathy, but, on the whole, the most significant additions to an understanding of the disease have been derived from investigations of the localization of lead in the tissues.

Another type of investigation which has proved of great value in lowering the incidence of lead poisoning has been a study of public

and industrial hygienic conditions. This has resulted in the installation of various protective measures which have greatly reduced the incidence of toxic manifestations. Probably the first public health work of this type was the elimination of the colic of Poitiers by preventing contamination of wine. In 1860, Lefèvre (251) pointed out that the dry colic observed on ships and in the French colonies was caused by lead, and insisted that precautions be taken. As a result the colic largely disappeared. Most of such work, however, has been done within the last twenty-five years, for the most part by Sir Thomas Oliver (337) and Legge and Goadby (252) in England, Meillère (293) in France, Teleky (455–459) in Germany, and Alice Hamilton (187–195) in America. Throughout these countries the passage of many regulations to safeguard workers and give them compensation for injuries has resulted. Both Cantineau (65) and Hutton (222) have recently summarized well the present legislative situation.

Requirement of prophylactic measures and modern knowledge of the action of lead have greatly reduced the incidence of industrial lead intoxication, particularly within the last ten years. With proper precautions it should be possible to control lead poisoning completely, and thus avoid the need of eliminating the use of lead in industry.

Part I. Chemistry

II. The detection and estimation of lead in organic material

The value of a satisfactory method for the detection of lead has been recognized for centuries, and various reactions have been suggested as the basis for suitable tests. Because of the general use of metallic lead and later of litharge or lead acetate to improve poor wine by correcting the harsh acid taste, general interest in this subject was manifested early. In Germany such disastrous results followed this adulteration of wine that decrees were issued in 1498 and 1577 which invoked the death penalty for adding lead to wine. Since then the ever widening interest in lead poisoning has resulted in many attempts to discover an easy and accurate method for the detection of lead.

One of the first of these was to add sulphuric acid to wine and to ascertain the presence of lead by the formation of lead sulphate—a white precipitate. In 1707 Zöller advocated the use of an extract of orpiment and lime water, which contained soluble sulphides and therefore denoted the presence of lead by the formation of a black precipitate. Later, in 1784, Bergman (33a) mentioned marine (hydrochloric) and vitriolic (sulphuric) acids and sal sodae (sodium carbonate) as precipitants for lead in the analysis of water, and stated that "a brown or black sediment indicates the presence of sulphur in a state of solution," if lead is added. Fourcroy and Hahnemann at about the same time suggested that acidified water saturated with hydrogen sulphide gas might be a suitable reagent. Thus, it is to lead that hydrogen sulphide gas owes its place in analytical chemistry. Gradually, as quantitative methods developed and chemical analysis became more exact, many other tests for lead were found. A comprehensive review of these was published by Rüdisüle in 1914 (392a).

Since in the examination of organic material only very small amounts of lead are involved, precautions, which are not necessary in dealing with larger quantities of lead, must be employed. Definite knowledge of the conditions under which a lead salt is precipitated is a prime requisite, for the formation of plumbites in the presence of excess sodium or potassium hydroxide renders soluble a number of lead compounds which are insoluble in water alone. Ammonium hydroxide precipitates lead hydroxide, but when present in excess does not dissolve the lead salt. Lead sulphide is insoluble in neutral and alkaline solution and is only slightly soluble in dilute acid. It is also relatively insoluble in hydrochloric acid, and the degree of acidity must be carefully adjusted when small amounts of lead are involved, if the lead is to be reprecipitated as sulphide, particularly in the presence of calcium chloride. In alkaline solution most lead salts are converted to basic salts, some of which are of undetermined composition. Table 1 is a list of the most insoluble of the lead salts. During fine manipulations it must be remembered that filter paper adsorbs lead salts readily when they have a neutral or slightly alkaline reaction. Still another possible source of error is to be found in the fact that at very high temperatures lead salts may volatilize (lead chloride boils at 900°C.) and thus be lost from the substance analyzed, as for example, in ashing organic material prior to chemical examination.

In other ways, too, the presence of organic material in substances to be analyzed for small amounts of lead adds complications, for many organic substances mask partially if not completely the ordinary reactions employed in tests for lead. For example, lead precipitates readily from aqueous solution as the chromate, but when serum is present the addition of chromate produces no visible change. Similarly, hydrogen sulphide produces a black precipitate in aqueous solutions of lead, while in serum containing the same quantity of lead it merely causes the appearance of a wine red color. To avoid such errors, organic matter must be completely destroyed. This is a delicate operation and if losses are to be prevented, manipulation must be extremely careful. With the proper technique, however, exceedingly small amounts of lead—1 part in 10,000,000 in urine, for instance—may be accurately detected. As investigations of the toxicity of lead have progressed, methods of analysis of material containing small amounts of lead have been refined until it has become evident that the methods which are exact for inorganic compounds are inaccurate when applied to organic substances.

The colorimetric sulphide method, which Pelouze (355a) first introduced in 1842, for detecting minute quantities of lead has long been a favorite. It is based upon the fact that since lead sulphide is very insoluble, the degree of color in a solution of soluble sulphides should be an index of the amount of lead present. As various interfering features have been discovered from time to time this method has been changed, for its great sensitiveness has made it seem valuable. It does not, however, give definite proof of the presence of lead because many other substances produce exactly the same coloration. It is interesting that even as early as 1814 Orfila (344) reported that urines uncontaminated with lead often gave a positive test. On this account, and also because both the size of particles and the concentration and type of salts and acids present affect the accuracy of the test markedly, other methods of analysis have supplanted it. In these, lead sulphide is still employed, but merely as a means of separating lead from other substances.

Another method which has been widely employed for the detection of lead makes use of electrolytic phenomena. When an electric current passes through a solution of lead salt in nitric acid, lead peroxide (PbO_2) separates from solution at the anode. This provides an excellent method of analysis for such relatively large quantities of lead as are present in ores

TABLE 1

Solubility of lead and its compounds

SUBSTANCE	SOLUBILITY	REFERENCES
	grams per liter	
Lead	1.2×10^{-2}*	Marais, H. Compt. rend. Acad. d. Sc. (1873) lxxvii, 1529
Lead bromide	8.342	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead carbonate	1.75×10^{-3}	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead carbonate (basic)	1.3×10^{-3}	Seidell, A. Solubilities of Inorganic and Organic Substances, N. Y., 1919
Lead chloride	10.708	Harkins, W. D. J. Am. Chem. Soc., 1911, xxxiii, 1807
Lead chromate	6.0×10^{-5}	v. Hevesy and Rona. Ztschr. phys. Chem. (1915), lxxxix, 303
Lead hydroxide	1.385×10^{-1}	Sehnal, J. Compt. rend. Acad. d. Sc., 1909, cxlviii, 1394
Lead iodide	4.70×10^{-1}	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead oxide PbO	6.87×10^{-2} 18°	Pleissner, M. Arb. a. d. k. Gsndhtsamte., 1907, xxvi, 402
Lead phosphate $PbHPO_4$	1.29×10^{-2}	Fairhall, L. T. J. Indust. Hyg., 1924, vi, 160. Lead Studies X.
Lead phosphate $Pb_3(PO_4)_2$	1.35×10^{-4}	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead sulphate	8.24×10^{-2} 20°	Sehnal, J. Compt. rend. Acad. d. Sc., 1909, cxlviii, 1394
Lead sulphide	8.6×10^{-4}	Weigel. 1907. Quoted by Seidell, p. 365

* In water saturated with CO^2.

or alloys, but unless scrupulous care is employed in the analysis of biological material, the method may lead to inaccurate results. Meillère (293) examined with this method the tissues and excreta of individuals poisoned by lead. Because he was fully cognizant both of the difficulties and errors involved and of the extent to which these might influence the results, his work admits of no criticism.

Denis and Minot (94) have devised a more exact electrolytic method. In this, lead is first separated as the sulphide from a solution made alkaline with ammonium sulphide. This lead precipitate is then washed with N/10 hydrochloric acid, the residue dissolved in nitric acid, and finally electrolyzed. The lead peroxide deposited at the anode (in this case a platinum dish) is next washed and treated with potassium iodide in acid solution. Since iodine is quantitatively liberated under these conditions, titration with sodium thiosulphate solution indicates the quantity of lead present. Minot (306) has very recently subjected this test to severe criticism. Washing the precipitate of sulphide with hydrochloric acid causes measurable loss of lead. Manganese in the substances analyzed may render results ambiguous by migrating to the anode as manganese dioxide and setting free iodine from the potassium iodide solution in acid medium. For instance, in the feces of a painter suspected of having lead poisoning, the quantity of manganese deposited in this way corresponded to 1.37 mgm. of lead; while the filtrate containing the acid washings was found by the chromate method to contain 0.29 mgm. of lead. Thus, unless extreme care is exercised at every stage in this test, conclusions may be very erroneous. Furthermore, the difficulties of manipulation necessitate great technical skill if minute quantities of lead are to be accurately measured by this method.

Detailed discussion of the various other methods of determining minute amounts of lead in organic material is needless. After careful investigation the chromate method has proved to be the most reliable, for the processes involved purify the lead to some extent and final precipitation occurs under conditions which allow insoluble salts of no other metal to form.

The chromate method. *Ashing.* Before analysis tissues and feces must be freed from water by baking. This may be done very rapidly by heating the material in porcelain dishes (Coors) on a hot plate until it starts to char. It should then be transferred to an electric or gas muffle furnace and ashed at dull red heat. Fecal matter usually ashes readily but the tissues form a

residue which must be repeatedly extracted before the entire char is consumed. Usually most material requires re-ashing as a certain quantity of inorganic salts becomes fused and prevents complete oxidation. After the first ashing the material should be cooled and extracted with dilute hydrochloric acid and hot water. It is essential that at this stage *all* the ash be dissolved, for frequently lead phosphate is present as an insoluble residue that might be mistaken for silica. If this residue is insoluble in hydrochloric acid it should be treated with a mixture of hydrochloric and tartaric acids (which dissolve lead phosphate), until the ash is quantitatively dissolved.

Precipitation as chromate. The strongly acid solution should be neutralized with NaOH. HCl should then be added until it is just acid to methyl orange. The next step is to saturate the cold solution with hydrogen sulphide. If sulphides precipitate to any great extent during this process they may be filtered at once, but if no precipitate appears the solution, saturated with hydrogen sulphide, should be allowed to stand over night before filtration. Immediately after filtration the precipitate should be washed, as lead sulphide oxidizes rapidly when in contact with air. Solution of the washed precipitate in nitric acid, boiling to expel hydrogen sulphide, cooling, and finally neutralization with sodium hydroxide as indicated by phenolphthalein are then necessary. After re-acidification with acetic acid, two or three drops of a saturated solution of potassium chromate should be added to the resulting solution. If the solution is held against a dark background during this process a slight turbidity may be observed around the drop of added chromate in the presence of even very minute quantities of lead. To hasten the reaction the solution should next be boiled for a few minutes. If no turbidity is apparent, the solution should then stand over night before filtration to allow separation of the extremely small amounts of lead chromate which it may contain. After filtering, all trace of soluble chromate should be washed from the filter paper, the chromate dissolved in a little dilute hydrochloric acid, an excess of potassium iodide solution added at once, and the free iodine titrated with 0.005 N sodium thiosulphate solution, a drop or two of starch being added near the end-point as indicator.

Preparation and preservation of standard thiosulphate solution. If the standard dilute solution of sodium thiosulphate is to remain stable for any length of time it must be kept under special conditions and must be prepared with special care. All distilled water should be freshly distilled and collected while still hot. Glassware must be chemically clean before coming into contact with the solution. In the large (20 liter) bottle which has been found most satisfactory for storing the solution, about 25 grams

of sodium thiosulphate should be thoroughly mixed with 18 liters of distilled water. The bottle should then be connected with the apparatus sketched in figure 1 to prevent decomposition of the thiosulphate. The two bottles, *D, D*, contain thiosulphate solution of the same strength as the

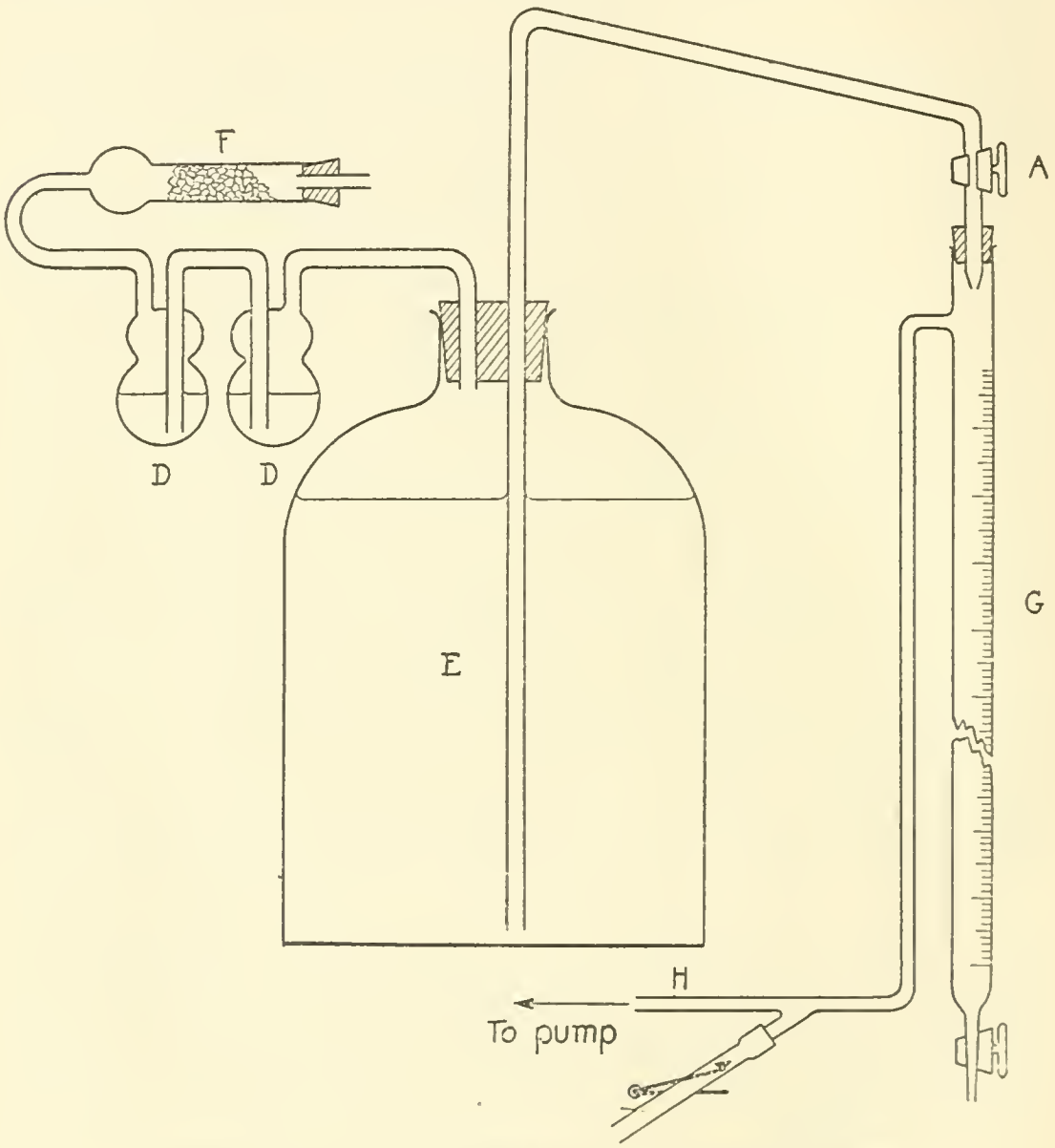


FIG. 1. APPARATUS FOR STANDARD THIOSULPHATE SOLUTION

stock solution, and are arranged to allow expansion and contraction of the air in bottle *E* without forcing the wash liquid into the soda lime tube *F*. The purpose of *F* is to remove carbon dioxide. If the cock at *A* is closed except when the burette *G* is being filled, no air can work its way back into the bottle *E*. With *H* connected to a suction pump the burette *G* is readily

filled by depressing key *B* (which fits over a piece of rubber tubing) and opening cock *A*. With this arrangement the burette may be filled rapidly and air can reach the stock solution only through *F*. In this way a solution of sodium thiosulphate (with a factor of 0.00558) has been kept for six months with its factor unchanged. If prepared with ordinary distilled water, however, and kept without protection from CO_2 the solution changes markedly within a few days. The titer of the thiosulphate solution should be determined twenty-four hours after preparing, and once a week thereafter in order to ascertain whether the solution is stable or undergoing decomposition. One cubic centimeter of 0.005 normal sodium thiosulphate solution is equivalent to 0.3451 mgm. of metallic lead.

Sodium thiosulphate can be standardized conveniently against (approximately) 0.005 N iodine which has been accurately standardized against 0.01 N arsenious acid (465). The arsenic tri-oxide used in preparing the arsenious acid should be purified by Chapin's method (75) because the arsenic tri-oxide designated "C.P." or "Analyzed" frequently contains large amounts of antimony tri-oxide.

Analysis of urine. In analyzing urine for lead, the procedure heretofore has been to evaporate to dryness, char and ash the residue. Since urine residues are particularly difficult to ash because of the large quantity of inorganic salts present, repeated extraction and ashing is necessary if all the salts are to be dissolved. This makes the process burdensome. The following new method in which evaporation is avoided by precipitating lead directly from urine has therefore been devised (130).

Ammonium hydroxide is added to urine until it is strongly ammoniacal. This mixture is allowed to stand from one to twenty-four hours. In this reaction the earthy phosphates are precipitated and lead phosphate is carried down quantitatively by *entrainment*. The gelatinous mass of phosphates settles into a compact mass from which the clear lead-free liquor may be decanted and the remainder rapidly filtered by suction on a Büchner funnel. The filter paper containing the precipitate ashes rapidly in a few minutes and the quantity of lead may then be determined by the chromate method outlined above.

The microchemical detection of lead. Very frequently the amount of lead is so minute (a few one-hundredths of a milligram) that the possibility of quantitative estimation is questionable, and under

these conditions merely the presence or absence of lead can be determined. A rapid qualitative test is therefore necessary. Very minute amounts of lead may be separated and detected microchemically (127) as the hexa-nitrite of potassium, lead and copper—$K_2CuPb(NO_2)_6$—a salt which crystallizes in black cubes or appears as reddish plates when seen in thin sections (figs. 2 and 3).

In this test the necessary reagents are: Sodium acetate 4 per cent, copper acetate 2 per cent, and acetic acid 10 per cent. The capillary pipette for measuring may be prepared by drawing out a piece of 4 mm. glass tubing

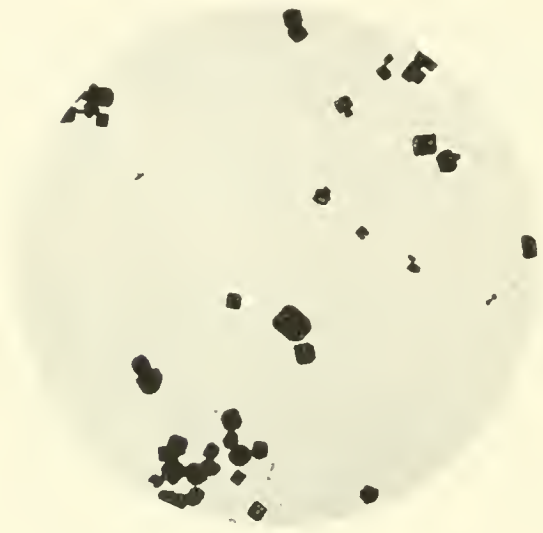


FIG. 2

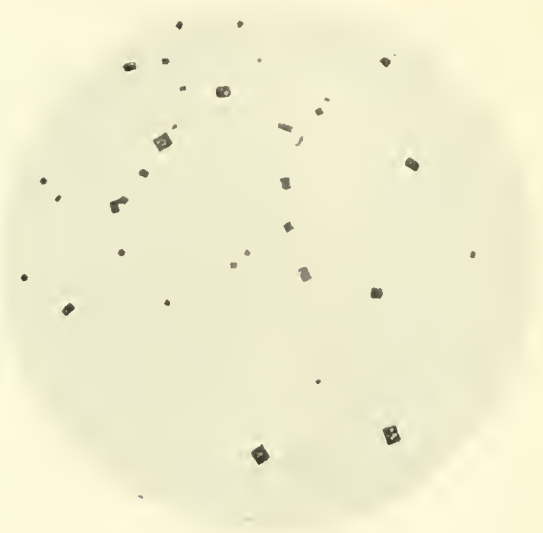


FIG. 3

FIG. 2. SHOWING LARGE REGULAR RECTANGULAR PLATES OR CUBES OF HEXANITRITE CRYSTALS. × 85

FIG. 3. SHOWING SMALL REGULAR RECTANGULAR PLATES OR CUBES OF HEXANITRITE CRYSTALS. × 350

to a capillary, blowing a bulb at one end, and calibrating the stem roughly by marking two points to represent a volume of 5 cmm. (about 1/10 of a normal drop). Filtering tubes may be made from glass capillary tubes with a tiny plug of absorbent cotton at one end. In order to precipitate the lead, the ash from any material should be dissolved in hydrochloric acid, neutralized and re-acidified with hydrochloric acid until just acid to methyl orange. One cc. of a saturated solution of ammonium sulphate and one drop of the 2 per cent copper acetate solution should then be added to the material in a centrifuge tube, placed in a beaker of ice and saturated with hydrogen sulphide gas. The total volume of the solution should be about 15 cc. After precipitation, the sulphides should be separated by centrifu-

galization and washed at least three times by decantation. After each washing the water must be completely drained from the sulphide precipitate by a capillary tube. The success of this test depends a great deal upon the thoroughness with which the soluble inorganic salts are removed by washing, as they interfere very much with the test. The next step is to drain the centrifuge tube containing the sulphides free from water, place it in a beaker of boiling water, and add two drops of nitric acid. Some of the solution thus obtained may then be drawn up into a capillary tube from which a drop or so is placed on a microscope slide. This should be evaporated to dryness. Five to 10 cmm. of 4 per cent sodium acetate solution should then be added and the residue completely dissolved. Occasionally when bismuth is present as a result of medication with bismuth salts, a

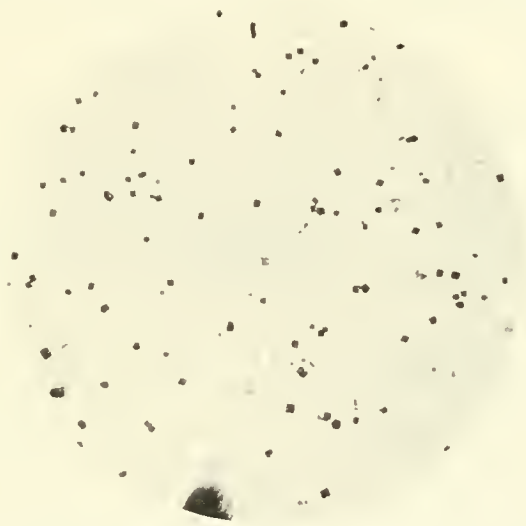


FIG. 4. SHOWING THE HEXANITRITE CRYSTALS FORMED IN THE PRESENCE OF A SMALL AMOUNT OF BISMUTH. × 350

whitish precipitate will form at this point. If this occurs, the acid nitrate solution in the centrifuge tubes should be evaporated to dryness and several drops of distilled water added. This precipitates the bismuth as bismuth oxy-nitrate which may be removed by dropping the glass capillary filtering tube into the mixture. The clear solution rising through the cotton plug contains a small amount of bismuth but not enough to interfere with the test for lead (fig. 4). The material should next be gathered together into one droplet and again evaporated to dryness on a slide. If this is carefully done the residue consists of a glazed rim about 4 mm. in diameter in which the greater part of the salts are concentrated. The slide should next be chilled on ice and 5 cmm. of a solution of 10 per cent acetic acid and a small crystal of potassium nitrite added. This acid and nitrite crystal should be

centrally placed on the dry residue and allowed to diffuse to the outer edge. Such slow diffusion permits the hexa-nitrite crystals to form at various places under optimum conditions of concentration. As the reagents, particularly potassium nitrite, often contain noteworthy amounts of lead, it is important to establish their purity either by re-crystallization or by the elimination of lead as sulphide wherever possible.

Summary. Of the various methods of determining minute amounts of lead in biological material, the chromate has been found to be the most accurate and satisfactory. For the mere detection of lead, a modified microchemical method has been developed. The analysis of urine for lead is greatly facilitated by a preliminary *entrainment* precipitation of lead phosphate with phosphates of the alkaline earths.

III. PHYSICO-CHEMICAL BEHAVIOR OF LEAD COMPOUNDS

While lead is both tetra-valent and di-valent, probably only the di-valent compounds are of physiological interest, with the one exception of lead tetra-ethyl which has attained some commercial importance. The transformations of lead salts within the body are probably few and consist largely in the conversion of insoluble salts into a soluble form and in the reconversion of absorbed lead into an insoluble salt for storage. Certain factors, such as degree of acidity and length of time of contact with the body fluids, govern the amount of lead that may enter the system after the ingestion of insoluble lead salts. Thus lead oxide can enter the system more readily than lead sulphate or lead sulphide because of its greater solubility in the digestive juices.

The lead compounds which most commonly enter the gastro-intestinal tract are the oxides, the sulphide, carbonate, and chromate.

Carlson and Woelfel (69), who determined the solubility of some of these compounds in gastric juice found that lead carbonate is slightly more than twice as soluble as lead sulphate. In their experimental work with animals lead carbonate proved much more toxic than lead sulphate, but both salts eventually caused acute symptoms of lead poisoning when administered in doses of 0.1 gram per kilo of body weight per day. Lead sulphide, both natural and synthetic, although less soluble than either of these salts, is,

nevertheless, absorbed in sufficient quantities to be dangerous. In a given amount of gastric juice the solubility of the carbonate is 46 per cent; that of the sulphate 9.5 per cent; and that of the natural sulphide 2.5 per cent.

Lead chromate, the pigment commercially known as chrome yellow, Paris yellow, or Leipzig yellow, which is employed for calico printing or dyeing, is generally considered one of the most toxic of the lead salts. It dissolves readily in dilute hydrochloric acid and consequently in the gastric juice. Beck and Stegmüller (29) have observed that while in dilute acid the solubility of this salt is almost proportional to the hydrogen ion concentration, in concentrated solution the quantity dissolved is proportional to the square of the hydrogen ion concentration. This is because in the latter case dichromate ions are formed.

$$Pb^{++} + CrO_4^{=} + 2H^+ + 2Cl^- \rightleftarrows Pb^{++} + 2Cl^- + H^+ + HCrO_4^- \qquad (1)$$

$$2HCrO_4^- \rightleftarrows Cr_2O_7^{=} + H_2O \qquad (2)$$

The second of these reactions so shifts the equilibrium that more and more lead is dissolved. The following equilibrium constants make this even more apparent by numerically indicating the interrelationship of the various types of reaction and the direction in which the changes will occur.

$$(1) \quad \frac{(H^+)\ (CrO_4^{=})}{(HCrO_4^{=})} = 3.7 \times 10^{-7} \qquad (3) \quad \frac{(H^+)\ (Cr_2O_7^{=})}{(HCr_2O_7^-)} = 1.0 \times 10^{-3}$$

$$(2) \quad \frac{(H^+)^2\ (CrO_4^{=})^2}{(Cr_2O_7^{=})} = 3.4 \times 10^{-13} \qquad (4) \quad \frac{(HCrO_4^-)^2}{(Cr_2O_7^{=})} = 2.5$$

Auerbach and Pick (22) further found that in the presence of excess sodium hydrogen carbonate, even the very insoluble salt, lead chromate, is converted into the carbonate, as illustrated in the following equation.

$$PbCrO_4 + 2NaHCO_3 \rightleftarrows PbCO_3 + Na_2CrO_4 + H_2O + CO_2$$

In order to determine what possible solvent action pancreatic juice might have upon these compounds, the action of sodium carbonate and sodium hydrogen carbonate upon the carbonate and sulphate of lead has been carefully studied by Auerbach and Pick (21).

When the total sodium concentration is below 0.077 N the reaction with sodium carbonate is:

$$3PbCO_3 + Na_2CO_3 + 2H_2O \rightleftarrows 2PbCO_3 \cdot Pb\ (OH)_2 + 2NaHCO_3$$

Above this point, however, the double salt, basic sodium lead carbonate ($NaPb_2(CO_3)_2OH$), is formed. Lead sulphate reacts with sodium hydrogen carbonate according to the equation

$$PbSO_4 + 2NaHCO_3 \rightleftarrows PbCO_3 + Na_2SO_4 + CO_2 + H_2O$$

The reaction is reversible and therefore depends upon the CO_2 concentration. It is possible that the reaction with sodium hydrogen carbonate may occur to some extent in the lungs where the particles are bathed in a medium containing small amounts of this salt, and thus account for the fact that lead chromate, although very insoluble, enters the circulation readily from the respiratory tract. It is evident therefore that very insoluble lead salts may be slowly converted into lead carbonate when in contact with pancreatic

TABLE 2

The solubility of various lead compounds in blood serum

NUMBER	SUBSTANCE	SOLUBILITY IN SERUM AT 25°C.	SOLUBILITY IN H_2O
		grams per liter	*grams per liter*
1	$PbCO_3$	0.0333	0.0017 $^{18°}$ (1)
2	$PbSO_4$	0.0437	0.044 $^{24,95°}$ (2)
3	$PbCrO_4$		0.00001 $^{25°}$ (3)
4	PbO	1.1520	0.0171 $^{20°}$ (1)
5	Pb	0.578	

(1) Pleissner, M.: Chem. Centr.-Bl., 1907, ii, 1056.
(2) Böttger, W.: Ztschr. f. phys. Chem., 1903, xlvi, 604.
(3) (von) Hevesy, C.: Ztschr. f. anorg. Chem., 1913, lxxxii, 328.

juice or with such other body fluids as blood plasma. While lead carbonate itself is somewhat insoluble, it is far more soluble than many lead compounds from which it can thus be formed; and the chances of its entering the circulation are therefore relatively great.

While the entrance of lead or its compounds into the gastro-intestinal tract is an important factor in the etiology of plumbism, absorption from the lungs produces lead poisoning much more directly (see section V). In industry, workmen are commonly exposed to the dust of white lead, of lead oxides, or of sandpapered paint. Fine particles of these various compounds are inhaled and very soon after becoming lodged in the alveoli may produce severe lead poisoning. Phagocytosis (137) and direct absorption of dissolved lead compounds, as well as gradual diffusion from slowly dissolving particles in contact

with the moist epithelial cells of the alveoli must play some rôle in this process. While metallic lead and lead oxide are strikingly soluble in blood serum (128) and lead carbonate is more soluble than in pure water, the solubility of lead carbonate in serum saturated with CO_2 is about as low as that in 0.005 N H_2CO_3 solution as determined by Pleissner (359).

The marked solubility of lead oxide and metallic lead in serum is significant, for in the lead industry poisoning develops rapidly after exposure to lead fume and to lead oxide dust. Legge and Goadby (252) point out that the incidence of lead encephalopathy is higher among people who manufacture lead oxides than in any other lead industry. Therefore, the chemical transformations that lead salts may undergo in the body assume an important and very practical aspect.

TABLE 3

Effect of CO_2 on the solubility of lead compounds in serum

NUMBER	SUBSTANCE	SOLUBILITY IN CO_2-SATURATED SERUM
		grams per liter
1	$PbCO_3$	0.0125
2	$PbSO_4$	0.0300
3	PbO	1.1600
4	Pb	0.1930

Since lead is slowly accumulated in the body and deposited in the bone tissue, probably as a phosphate (see page 25) some knowledge of the reactions and equilibria involved in the formation of the phosphates of lead is necessary. The conditions under which these phosphates form at room temperature are not very well known and most investigations have been carried out only under synthetic rather than equilibrium conditions.

Investigation (126) has demonstrated that under equilibrium conditions di-sodium phosphate and lead chloride react to form di-lead phosphate, $PbHPO_4$. This occurs only under equilibrium conditions, for when the two reacting substances are first mixed, tri-lead phosphate is formed. The curve (fig. 5) illustrating the changes in hydrogen ion concentration with increasing amounts of sodium phosphate shows that the acidity rises to a

maximum and then decreases. If the two substances react, as is generally stated, according to the equation,

(a) $$PbCl_2 + Na_2HPO_4 \rightleftarrows PbHPO_4 + 2NaCl$$

the solution should remain neutral, whereas if tri-lead phosphate is formed,

(b) $$3PbCl_2 + 2Na_2HPO_4 \rightleftarrows Pb_3(PO_4)_2 + 4NaCl + 2HCl$$

it should become acid.

In figure 5 it may be seen that the solution becomes more acid with each successive addition of di-sodium phosphate until there is present nearly

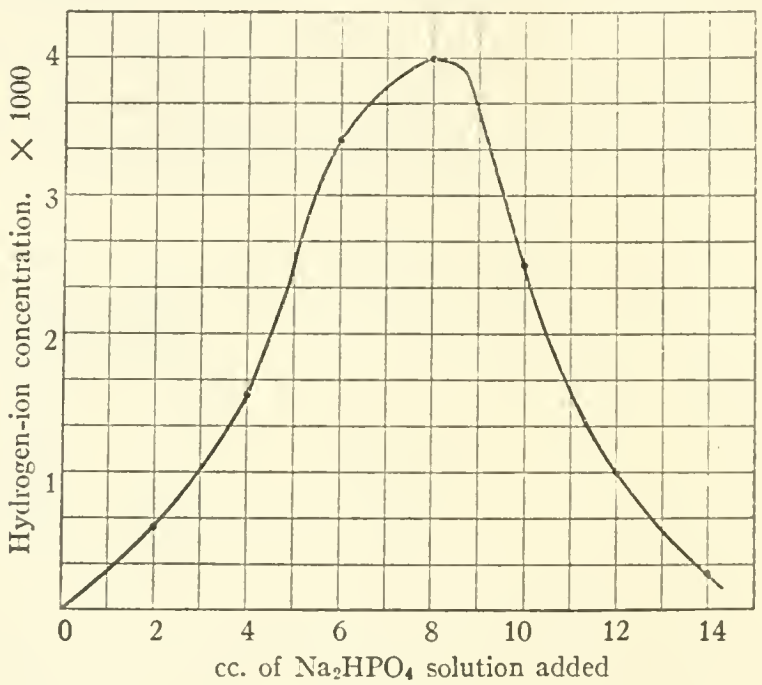


FIG. 5

the amount of phosphate theoretically necessary to precipitate the lead as tri-lead phosphate. Although under these conditions the amount of sodium phosphate is only 66 per cent of that required to precipitate the lead as di-lead phosphate, the filtrate is lead-free. From this point on, the acidity decreases because of the excess sodium phosphate. Thus it is evident that under *synthetic* conditions or in slightly alkaline solution the tri-lead is formed, while under *equilibrium* conditions di-lead phosphate is formed.

The transformation of di-lead phosphate into the tri-lead salt takes place in the following manner:

$$3PbHPO_4 \rightleftarrows Pb_3(PO_4)_2 + H_3PO_4$$

when the salt is suspended in boiling water. A constant increase in the

acidity of the solution, as well as the change in microscopic appearance of the salt, is evidence of this reaction. This change is very slow and requires several hours even under the most favorable conditions.

The phase diagram in figure 6 shows the range of existence of lead phosphates over a wide range of acidity. In the area *ACE* solution is complete and no solid phase is present. Experiments 1 to 10 inclusive are plotted along the straight line *AC*. The lines connecting these two points with

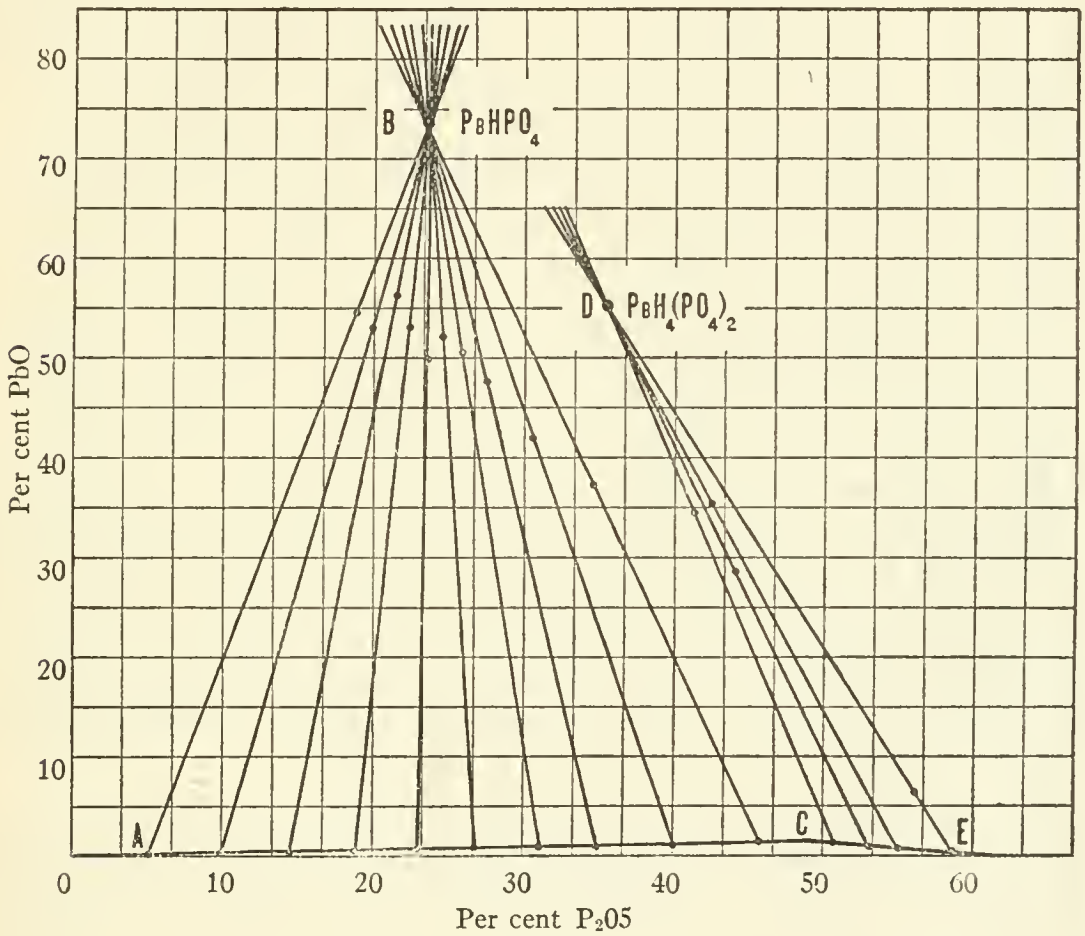


FIG. 6

those representing the composition of the solid residues, converge at *B* which represents the compound, di-lead phosphate. As these experiments include a wide range of concentration, this point is quite satisfactorily established. The area *ABC* therefore represents a mixture of the solid phase with various solutions. At *C* there is a maximum amount of lead in the solution phase. This point, the intersection of the two branches *AC* and *CE*, falls where a constant solution would exist in contact with the two phases di-lead hydrogen phosphate and mono-lead tetra-hydrogen

phosphate. The branch *CE* (experiments 11 to 14) illustrating the solid phase $PbH_4(PO_4)_2$ in contact with varying solutions, and the lines representing the compositions of the solution phase $PbH_4(PO_4)_2$ in contact with varying solutions, and the lines representing the compositions of the solution phase and solid phase very nearly coincide with point *D*, representing the theoretical composition of $PbH_4(PO_4)_2$ when they are projected.

Thus the phase diagram indicates quite clearly the stability of the di- and mono-lead phosphates at various acid concentrations and furnishes means of predicting the result of bringing various mixtures to equilibrium. Any mixture represented by points within the area *ACE* for example must yield a solution with no solid phase. The composition of any compound formed by the empirical mixture of substances falling within the limits of the area *ABC* may be similarly foretold. The short branch *CE* illustrates the solubility of mono-lead tetra-hydrogen phosphate in phosphoric acid at 25°C. and shows that this rapidly decreases as the concentration of acid increases.

Since tri-lead ortho-phosphate cannot exist at equilibrium in even slightly acid media, the behavior of lead within the body is the more easily explained. While lead phosphate under the normal conditions of alkalinity remains inert and harmless, any *local* production of acid must transform the tri-lead salt to di-lead phosphate. Since the solubility of the latter salt is 12.9 mgm. per liter and that of the tri-lead is only 0.13 mgm. per liter, the solubility of lead is thus increased an hundred-fold.

This shows, therefore, that the two lead phosphates of physiological interest are tri-lead and di-lead hydrogen phosphate. The transformation of one into the other is governed by conditions within the body and may have an important bearing upon the health of the individual. Indeed, this may account for the fact that lead may be stored in the body for years without causing any ill effects until some such influence as deficient diet or acute infection alters the reaction of the tissues and increases the amount of lead in circulation.

Summary. It is apparent from the foregoing discussion that the products of the reaction between secondary sodium phosphate and a lead salt are tertiary lead phosphate and acid. This lead salt is very stable at the normal hydrogen ion concentration of the body, but is

sensitive to slight changes in acidity and passes into the more soluble di-lead salt readily. The marked solubility of lead and lead oxide in such body fluids as blood plasma may account for the high incidence of lead poisoning among workers exposed to lead fume and lead oxide dust. Both gastric and pancreatic juice may transform insoluble lead salts into more soluble forms and may therefore cause an increased absorption of lead compounds.

IV. THE TRANSPORTATION AND DEPOSITION OF LEAD IN THE BODY

Transportation of lead in the body. Definite investigation of the form in which lead is transported in the blood stream is very difficult because during plumbism the amount present in the blood at any given time is so minute that isolation and identification are almost impossible. Certain considerations, however, suggest that whatever lead is present exists as the phosphate,—probably in colloidal form, rather than as the albuminate as Sir Thomas Oliver suggests (337). The chief of these is the fact that in plasma the proportion of lead to inorganic phosphate is very small. Because lead phosphate is extremely insoluble, any lead should therefore combine with the phosphate. Experiments (page 34) also indicate that lead is stored in the body as phosphate, while electrometric measurements (*vide infra*) in which lead salts were added to serum indicate that the lead is in combination with the inorganic phosphate group.

Although the phosphate is thus formed it does not precipitate from the blood serum when present in small amounts, but remains suspended as peptized or highly dispersed colloidal lead phosphate. Other lead salts behave in the same way when they are formed in serum. Lead chromate, for instance, apparently remains in solution. The fact that lead can be transported in the blood stream as an insoluble salt is therefore not surprising. When a clear solution of highly dispersed lead phosphate in serum is treated with hydrogen sulphide, part of the phosphate is changed to colloidal lead sulphide and although the solution remains perfectly transparent its color changes to a deep red. This shows that the sulphide is formed in a highly dispersed condition. Similar peptizing effects can be shown with substances other than serum. Thus, in the presence of cane sugar lead phosphate may be so highly dispersed that the solution is

as transparent as distilled water. If hydrogen sulphide gas is passed through such a preparation the change in color is similar to that already mentioned; here again there is no precipitation, for the lead sulphide remains highly dispersed and apparently in true solution. These facts lead one to believe (*a*) that lead could hardly be present in the blood stream except as lead phosphate, and (*b*) that it is carried in colloidal form (see also page 29).

Tests carried out with very refined technique have shown that only minute quantities of lead are present in the blood at any given time. For instance, the blood of animals which have been fed with lead salts usually contains only a few thousandths of a milligram of lead as long as the animals are in apparent good health. When such complications as acidosis develop and the animal becomes sickly, the quantity of lead in circulation increases slightly. In a case of fatal lead encephalopathy in man there were in the blood 2.8 mgm. of lead per liter—an amount which corresponds to 3.64 mgm. of tertiary lead phosphate. Since only 0.13 mgm. of this lead salt dissolves in a liter of pure water (246) the blood in this case contained about thirty times as much lead as could be dissolved in a corresponding volume of water. This is further evidence that *in vivo* lead is transported as colloidal lead phosphate.

That the greater part of the lead in the blood is held by the plasma and that but little is carried in the cells has been ascertained by experimentation. In a rabbit with lead poisoning the lead in the blood amounted to 0.75 mgm. of which 0.59 mgm. was in the plasma and 0.16 mgm. in the red cells. Thus, practically 80 per cent of the lead was carried by the plasma.

When a soluble salt of lead is added to blood serum, a precipitate, probably lead albuminate, an organic salt, forms at first and then dissolves. This is not particularly insoluble in water and is quite sensitive to changes in acidity. The maximum precipitation occurs at pH 5.8 which is probably the iso-electric point, for above or below this the salt re-dissolves. The precipitate is white and separates easily from solution. When dissolved in a few drops of dilute hydrochloric acid and treated with hydrogen sulphide gas, no change in color occurs until after a short time lead sulphide precipitates rapidly.

Since some authors have assumed that lead is carried in the blood

as an albuminate, the effect of the serum proteins upon the electrical behavior of dissolved lead salts is of interest. Measurements of the lead ion concentration should demonstrate whether the lead salt in solution is complex and whether the lead is in the anion. If sodium hydroxide is added to a solution of lead salt the lead hydroxide which at first precipitates is redissolved because of the formation of the complex sodium plumbite.

The successive steps are indicated in the following equations.

a) $$Pb^{++} + 2OH^- \rightleftarrows \underset{\text{solid}}{\underline{Pb(OH)_2}}$$

b) $$Pb(OH)_2 + NaOH \rightleftarrows NaHPbO_2 + H_2O$$

c) $$NaHPbO_2 \rightleftarrows Na^+ + HPbO_2^-$$

When an excess of sodium hydroxide is used, the positively charged lead ions (Pb^{++}) are wholly used up—the lead appears in the negatively charged group $HPbO_2^-$. Since in reaction (*b*) when there is excess of sodium hydroxide present, equilibrium lies in the direction of the formation of sodium plumbite, the lead ion (Pb^{++}) concentration must be enormously reduced. Electrometric measurements of the degree of this reduction may be made by determining the electromotive force of the concentration cell,

$$Pb \mid 0.1\ M\ KCl \text{ saturated with } PbCl_2 \mid NH_4NO_3 \text{ saturated} \mid 0.01\ M\ KCl \text{ saturated with } PbCl_2 \mid Pb$$

which is markedly increased by the addition of sodium hydroxide to one of the half elements of the electrical cell.

Without discussing the several factors that must be taken into account in making measurements of this character, the method of determination may be briefly described as follows:

Two beakers, one containing dilute, the other strong lead chloride solution, are connected by means of an inverted U tube filled with conducting salt solution. If a rod of pure lead is inserted in each, a so-called concentration cell is formed which will "run down," when the cell is short-circuited by connecting the two electrodes. During this process lead passes into solution in the beaker containing dilute lead chloride, acquires a positive charge, and the lead ion concentration increases. In the more concentrated solu-

tion lead ions give up their positive charge and there is a tendency for lead to plate out on the electrode. By means of a sensitive galvanometer introduced into the circuit, it is possible to demonstrate that the magnitude of the electromotive force depends upon the difference between the lead ion concentration in the two half cells or elements. As the lead ion concentration decreases in one of the beakers while that in the other remains constant, the electromotive force increases. Therefore, if the lead ion concentration (C) in one solution is known and the electromotive force of the cell is determined, the lead ion concentration (C') of the other solution may be computed from the Nernst equation $e.m.f. = 0.058\ ln\ \frac{c}{c'}$.

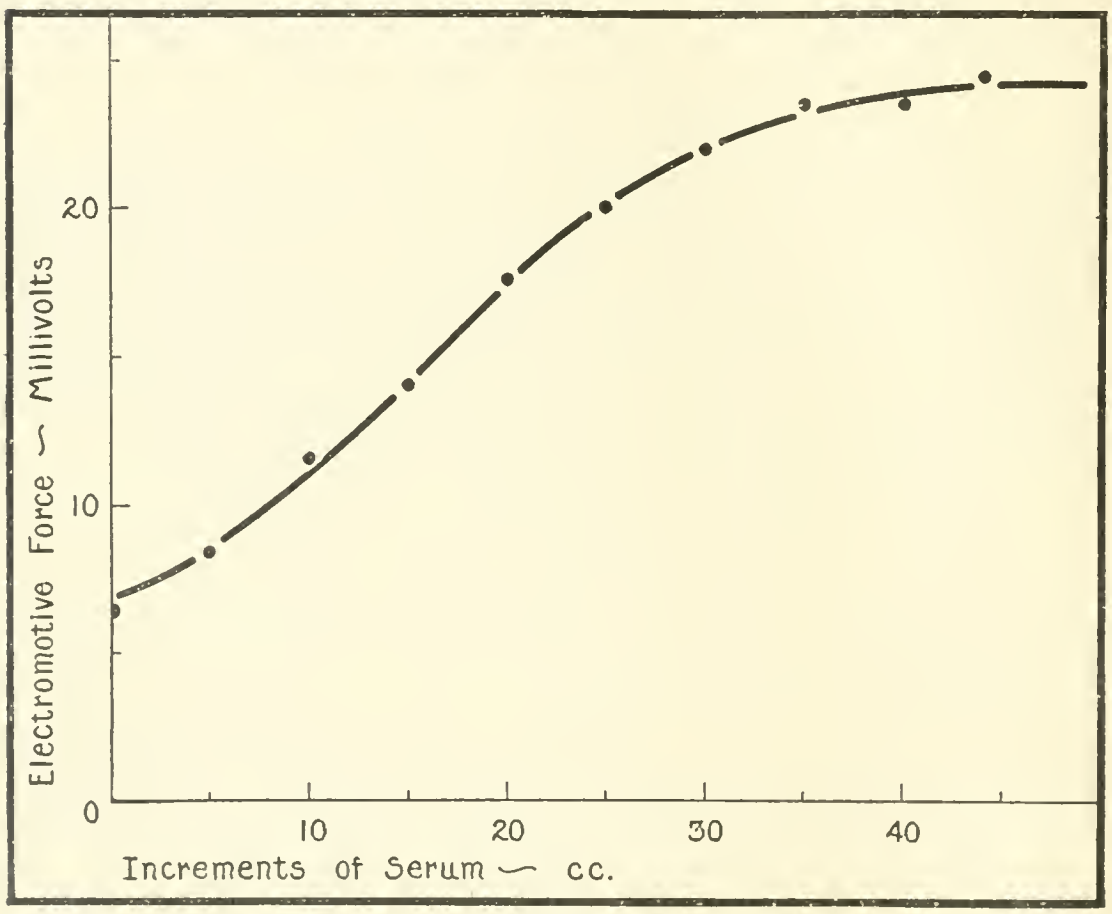


FIG. 6B

A typical experiment with serum was performed as follows:

Lead chloride in amounts containing from 1 to 10 mgm. of metallic lead was added to 0.1 M KCl solution containing 2 cc. of rabbit serum. These mixtures in volumetric flasks were then diluted with 0.1 M KCl to exactly 50 cc. With the various solutions thus prepared, amalgam cells were set up so that measurements of the electromotive force could be made at inter-

vals until equilibrium was apparently established. Similar dilutions of lead chloride alone in 0.1 M KCl were prepared for another series of amalgam cells to provide data on the extent of electrical dissociation of lead chloride. Amalgam cells containing 0.1 M KCl saturated with $PbCl_2$ were used as reference cells. When compared with a standard calomel cell the values checked favorably with those obtained by Lewis (263).

The experimental results are collected in table 4. From these figures it may be seen that the first decrease in lead ion concentration becomes less marked as the ratio of lead to serum increases. This is merely because at first some of the lead is thrown out of solution as a protein precipitate. With comparatively large amounts of lead (above 5×10^4 mols), the precipitate re-dissolves and the

TABLE 4

Effect of small amounts of lead chloride on serum proteins

TOTAL LEAD PRESENT $\times 10^4$ MOLS.	MEASURED LEAD ION CONCENTRATION $\times 10^4$ MOLS.	THEORETICAL LEAD ION CONCENTRATION $\times 10^4$ MOLS.	DIFFERENCE $\times 10^4$ MOLS.
1	0.05	0.95	0.90
2	0.60	1.50	0.90
3	1.90	2.25	0.35
4	2.44	2.60	0.16
5	2.80	2.80	0.00
6	3.08	3.09	0.01
7	3.20	3.20	0.00
8	3.53	3.53	0.00
9	3.70	3.75	0.05
10	3.90	4.00	0.10

lead is ionized normally. If the serum proteins and lead had combined to form a complex salt in which lead was present in the anion, the Pb^{++} ion concentration would have markedly decreased. Therefore we can state that there is no evidence that lead is carried in the blood stream as a complex salt in which the lead is in the anion.

When a small quantity of lead is titrated with a large quantity of serum, the results are different. The small initial precipitate dissolves at once in the excess serum, but the lead ion concentration decreases slowly. This is shown in table 4 and in figure 6B in which the increments of serum are plotted against the values for electromotive force which are inversely proportional to the lead ion con-

centration. The curve is somewhat S-shaped with its steepest inclination at the point where about 15 cc. of serum was added. This corresponds approximately to the point at which all the lead would be combined with the inorganic phosphates of the serum. These facts justify the conclusion that the reduction in the lead ion concentration is not due to combination of lead with the proteins of the blood but to its combination with certain of the mineral constituents of serum (probably the phosphate ions because the carbonate is more soluble) when a large excess of serum is present. No precipitate is visible because the lead phosphate is highly dispersed by the peptizing action of the serum proteins. All these facts, therefore, point to the conclusion that lead is transported in the blood stream as highly dispersed or colloidal lead phosphate.

The deposition of lead salts. An understanding of the conditions and reactions underlying the calcium exchange in bone is quite essential in a study of the deposition of lead salts in the skeleton. The early experiments of König (240) and the later work of Lehnerdt (258) and of Stöltzner (446) on the substitution of strontium for calcium demonstrate quite clearly that the calcium in bone tissue may be replaced by other elements with somewhat similar reactions. Until the properties of some of its compounds are known, however, lead would hardly be considered one of these, and the remarkable deposition of lead in bone tissue (see section VI) would scarcely be anticipated. Lead resembles calcium in that it forms both an insoluble carbonate and an insoluble phosphate. This lead phosphate is one of the few insoluble phosphates which does not react with most chemical reagents. Neither the mechanism of the deposition of lead in bone nor the process by which calcium is deposited are fully understood. Although certain conditions are known to retard the deposition of calcium while others increase it, the actual process by which calcium is laid down still remains obscure. Of the several theories advanced to account for calcium deposition, none is completely satisfactory.

Wells (488) has discussed some of the earlier theories, among them the conception that calcium fixation or deposition is the result of chemical reaction; and the hypothesis that calcium is first deposited as a soap which

later becomes transformed into the corresponding salts by contact with the carbonates and phosphates of the blood. Experimental work has neither confirmed this idea of soap formation nor demonstrated that cartilage is transformed into normal bone by passing through an intermediate soap stage; and Wells, after a careful study of this problem, has concluded that calcification is a process of adsorption of calcium by hyaline tissue which has a more or less specific affinity for calcium. Other hypotheses have been advanced more recently. Mellanby (295) has concluded from his study of rickets in children that the deposition of calcium in the bones is initiated by some accessory food factor. His experiments on puppies demonstrate that although rickets may be produced while they are very young, beyond a certain age the disease seldom develops, apparently because of a process of self-cure. The same seems to hold true for children. Beyond a certain age (two years) the development of active rickets is rare, probably because of the development of some essential process or secretion. Robison (389) has found that an enzyme in the ossifying cartilage of young rats and rabbits rapidly hydrolyses the hexose-mono-phosphoric acid ester present in small amounts in the plasma and in relatively large amounts in the corpuscles of the blood, and thus sets free phosphoric acid.

The theory that increasing the phosphate ion concentration ($HPO_4^{=}$) of the bones in this way would result in a local precipitation of calcium phosphate, is ingenious but does not explain the simultaneous deposition of calcium carbonate which is known to form 10 to 15 per cent of the inorganic constituents of normal bone. This brief review of the various theories shows how inexact is our knowledge of the mechanism of the deposition of calcium.

Perhaps the most stimulating hypothesis thus far suggested is that of Wells, i.e., that the deposition of calcium is to be attributed to an initial adsorption of calcium by the hyaline cartilage. In 1904 Pfaundler (358), by means of discs of gelatin, made a systematic study of the physical process of absorption of various crystaloids, and concluded that gelatin has a specific ionic affinity for calcium. Freudenberg and György (148) have recently conducted an extensive investigation based to some extent on this earlier work. They found that cartilage immersed in calcium chloride solution absorbed calcium in amounts which were regulated by the hydrogen ion concentration. The more acid the solution the less calcium was absorbed. They also found that cartilage removed magnesium, barium,

and strontium from solution in the same way. It is, however, apparent from their results that even when the time of contact was greatly prolonged absorption was not complete. Such calcification as this by chemical or physical combination of calcium and tissue protein is not, of course, calcification in the histological sense, for there is no such structural differentiation as appears in true calcified tissue. When metals like lead are fixed by cartilage, they penetrate in an even mass to various depths depending upon concentration, time of contact, etc. After this treatment cartilage presents a picture so entirely different from that of normal calcified tissue that the dissimilarity between the two is at once evident.

Histological examination of bone shows that calcium salts exist as such between the cells and not chemically bound to the protein. That the calcium is deposited in the intracellular spaces of the bone tissue as a secretion of the osteoblasts has been generally assumed by histologists (82). Quite recently Rabl (372) has been able to differentiate between calcium carbonate and calcium phosphate on the one hand and soluble calcium salts in calcifying tissues on the other. For this purpose he used ammonium oxalate because it reacts only with the dissolved calcium salts and not with the carbonate or phosphate. By this method the presence of dissolved calcium salts not only in the ground substance but also in the cells of growing bone tissue may be demonstrated, but without giving any clue as to the mechanisms of the absorption and deposition of calcium in the intracellular spaces, or the process by which calcium phosphate and carbonate are formed. In bone formation 85 to 90 per cent of calcium phosphate as compared with 15 to 10 per cent of calcium carbonate is deposited. This ratio is invariable and must depend upon rather accurately defined equilibrium conditions. These in turn are evidently regulated by the inorganic constituents of the blood plasma. The occurrence of a pathological increase of calcification in certain areas having an impoverished blood supply has been mentioned frequently (331), and this phenomenon may have an important bearing on the explanation of the various processes involved.

On the other hand, Tanaka has shown (452) that calcium phosphate injected in areas where the blood supply is relatively rich is absorbed with great rapidity; and (452) that if solid tri-calcium

phosphate is shaken for twelve days at body temperature in a solution containing the inorganic salts of the blood plasma (0.9 per cent NaCl, 0.1 per cent Na_2CO_3, and 0.1 per cent $NaHCO_3$) the calcium is bound—85.19 per cent as $Ca_3(PO_4)_2$ and 12.76 per cent as $CaCO_3$. This experiment *in vitro* is not conclusive because the solution was renewed daily and if continued sufficiently long all the calcium phosphate would be converted to calcium carbonate. In other words, there was no equilibrium established in a chemical sense. These experiments illustrate how difficult it often is to duplicate experimentally conditions or changes which occur within the body.

While lead resembles calcium in many respects—a fact which accounts for its deposition in bone tissue—there are several important differences between the two, particularly with regard to the solubility of certain salts. Lead phosphate is much more insoluble than lead carbonate, for instance, while calcium phosphate is more soluble than calcium carbonate. Therefore the mechanism which tends to precipitate the bulk of calcium in bone as calcium phosphate—the more soluble salt—would precipitate lead most readily as lead phosphate—a very insoluble salt. Again, calcium carbonate is sensitive to changes in carbon dioxide concentration, and it is not unlikely that the calcium stored in this form in the bones tends to regulate to some extent the amount of calcium in circulation. The solubility of $CaCO_3$ is only 9.6 mgm. per liter, whereas the solubility of $Ca(HCO_3)_2$ is 385 mgm. per liter. Furthermore, carbonic acid has been shown to exert a pronounced solvent action upon calcium phosphate (278), while it converts lead phosphate only very slowly to the di-lead salt. Fluctuations in the concentration of CO_2 would therefore regulate and keep fairly constant the quantity of calcium in the blood stream, whereas they would not affect the deposited lead salt.

Numerous experiments have been performed (129) to determine the composition of the lead salts stored in the body. Minot (307) has shown that storage occurs in the skeleton. Bones of animals experimentally poisoned with lead were therefore studied to determine the chemical composition of the deposited lead salt, for it had been previously suggested that lead is deposited in the body as an al-

buminate or carbonate. Both these salts are soluble in acetic or similar weak acids, but the repeated attempts made to dissolve lead from bone tissue with these acids have always resulted in failure. It is, however, possible to remove lead from bone with nitric acid in which lead phosphate is soluble. To determine the exact nature of the lead compound in bones is obviously very difficult, for the amounts of calcium phosphate and carbonate present are enormously greater than the quantity of lead deposited. Prévost and Binet (368) came to the conclusion in 1889 that lead was stored as phosphate because of its insolubility in sulphuric acid and ammonium acetate, and its solubility in nitric acid. Tartaric acid, which is a good solvent for lead phosphate, removes lead from bone tissue but dilute hydrochloric acid, which would dissolve lead only slightly, must be present to keep the insoluble calcium tartrate in solution. Sulphurous acid completely dissolves the calcium from bone as acid calcium sulphite, $Ca(HSO_3)_2$, but leaves the lead unaffected. Therefore lead in bone tissue is in the form of a less soluble salt than the carbonate or albuminate.

Because the amount of lead present is so minute—usually about 10 to 20 mgm. in 150 grams of bone—it has been impossible to separate the lead compound and to identify it definitely as lead phosphate. But experiments have been performed on the different lead salts which throw some light on the problem. When circulating lead comes into contact with the organic matrix of bone it is doubtless deposited much as is calcium except that it can be deposited in any quantity only as the phosphate. The solubility of lead phosphate in water is 0.13 mgm. per liter, while that of lead carbonate is 1.7 mgm. per liter. When precipitated as phosphate by di-sodium hydrogen phosphate, lead is in the form of the tri-lead salt which is transformed slowly in neutral or slightly acid solution into secondary lead phosphate ($PbHPO_4$). This di-lead salt always forms under equilibrium conditions (126) but in a slightly alkaline medium similar to that of the body, the tertiary salt is the stable form. It is in this very insoluble condition that lead is probably stored in the bones. Under normal conditions it may remain inactive for comparatively long periods, but when the alkalinity decreases, as in acidosis, the equilibrium swings toward the most soluble lead salts so

that lead may be set free (see section IX) and cause acute symptoms of lead poisoning.

Solubility determinations of di-lead phosphate prepared by the synthetic method (Series I) (4), and under equilibrium conditions (Series II) have been performed. The results are summarized in table 5. The average solubility (0.0129 ± 0.0004 gram per liter) proved to be approximately one hundred times that of tri-lead phosphate. This pronounced difference is important, for any variation in local reaction, such as increased acidity, may affect to a marked degree the quantity of lead in circulation. Even as weak an acid as carbonic can react slowly with tri-lead phosphate in the following manner:

$$Pb_3(PO_4)_2 + H_2CO_3 \rightleftarrows PbCO_3 + 2PbHPO_4$$

The solubility of this tri-lead salt in carbonic acid corresponds to that of the di-lead salt in pure water. When shaken for several hours at room temperature in water saturated with CO_2 the solution phase contained as much as 13.0 mgm. of lead phosphate per liter.

The failure of weak acids to dissolve lead *in vitro* from bones containing lead is undoubtedly in part dependent upon the buffer action of calcium phosphate and in part upon the decreased solubility of lead phosphate in the presence of excess phosphate ions. But a factor of probably greater importance than either of these is the fact that *in vivo* the production of acid is local and proceeds from the interior of the cell outward, whereas *in vitro* the action of the acid is from the outside in.

If normal and experimental conditions are considered from the viewpoint of hydrogen ion concentration, deficiencies of the experiments *in vitro* become more apparent. Local production of acid within the cell must raise the hydrogen ion concentration far more than can the mere addition of the same amount of acid diffused through a large volume of solution. Therefore lead in the bone must be more affected by local changes than it is by variations in the circulating medium which bathes the cells and by buffer action which maintains a nearly constant hydrogen ion concentration.

When tri-lead phosphate is shaken with lactic acid the phosphate neutralizes the acid (table 6 and fig. 7). Since lead lactate is soluble,

much more lead is carried into solution than when the lead salt is mixed with carbonic acid.

$$Pb_3(PO_4)_2 + 2CH_3CHOH\ COOH \rightleftarrows Pb(C_3H_5O_3)_2 + 2PbHPO_4$$

That local changes in reaction of the tissues are among the factors determining how much lead can pass into circulation, is therefore not unlikely. This solubility of tri-lead phosphate in lactic acid is particularly striking and may account for the fact (see section VI)

TABLE 5

Solubility of di-lead phosphate in water at 25°C.

SERIES I—SYNTHETIC $PbHPO_4$		SERIES II—EQUILIBRIUM $PbHPO_4$	
Grams per liter	Deviation from mean	Grams per liter	Deviation from mean
0.0134	0.0005	0.0132	0.0003
0.0120	0.0009	0.0120	0.0009
0.0129	0.0000	0.0143	0.0014
0.0130	0.0001	0.0124	0.0005
0.0132	0.0003	0.0125	0.0004
		0.0133	0.0004
0.0129	Average	0.0129	Average

TABLE 6

Solubility of lead phosphates in lactic acid at 25°C.

NUMBER	LACTIC ACID NORMALITY	DISSOLVED DI-LEAD PHOSPHATE	DISSOLVED TRI-LEAD PHOSPHATE
		gram per liter	*gram per liter*
1	0.047	0.141	0.686
2	0.093	0.197	1.175
3	0.186	0.283	1.794
4	0.465	0.458	2.296
5	0.930	0.484	2.661

that only traces of lead are found in muscle tissue, while large amounts may be present in bone.

The absorption of lead by bone. Experiments have shown that pieces of bone taken from an animal immediately after death absorb lead from a dilute solution of lead salt at a rate which depends upon the size of the bone particles. This lead is not removed by the formation of an insoluble albuminate, for the freer the bone from protein material the more efficiently is the lead taken from solu-

tion. These interesting facts have led to an investigation of the power of crushed bone to absorb lead.

In a series of experiments bone was carefully freed from fat by repeated extraction with chloroform and ether, and then from protein material by extraction with boiling salt solution and water until the biuret test was no longer positive. The crushed bone was carefully graded by sifting, and was shaken in the shaking machine with lead chloride solution at various concentrations. Because of the mutual grinding action of the bone particles under these conditions a certain amount of bone becomes more finely divided. It is, therefore, more satisfactory to allow the solution to flow continually over a bed of crushed bone. The apparatus shown in figure 8 was devised for this, and since it is automatic, uniform conditions may

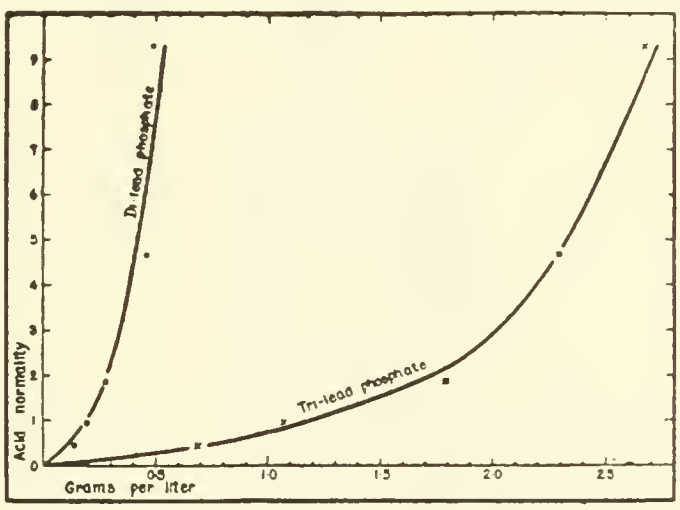


FIG. 7. SOLUBILITY OF LEAD PHOSPHATES IN LACTIC ACID AT 25°C.

easily be maintained. That lead is quickly removed from solution under such conditions is shown by table 7 and figure 9. The rate of removal is high—at the end of fifteen minutes the lead is completely withdrawn from solution. Such other substances as charcoal and cellulose which adsorb lead, or permutite which interacts with it chemically, are relatively ineffective in removing lead from solution when compared with crushed bone.

To demonstrate that lead is removed from solution by replacement of the calcium in the calcium phosphate of bone, required but little experimentation. Lead solutions of known concentration were pumped over crushed bone until the lead was completely absorbed. The filtrate containing the calcium was then analyzed and the amount of calcium liberated compared with the amount of lead absorbed. Table 8 and figure 10 indicate that the

amount of calcium liberated increases as larger quantities of lead are absorbed. The process is, therefore, one of replacement.

The degree of absorption of lead by crushed bone depends upon the hydrogen ion concentration (table 9 and fig. 11). Lead is not

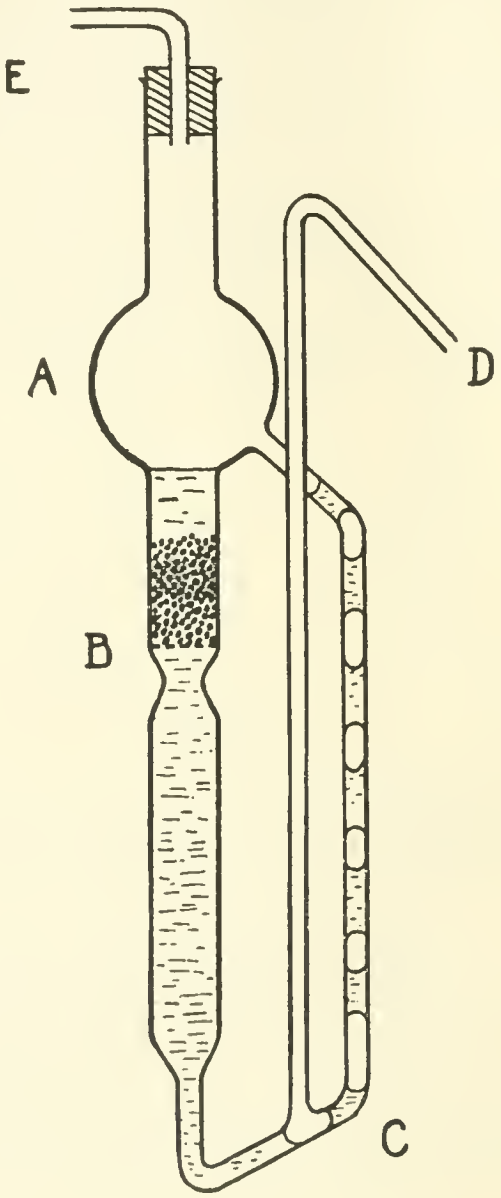


FIG. 8. CIRCULATING PUMP

removed very effectively from the more acid solutions, but as the acidity falls lead absorption increases until at pH 7.8 the maximum absorption occurs. Below this point lead is again absorbed in decreasing amounts. It is of biological interest that the range of maximum absorption *in vitro* lies between pH 7.4 and 7.8.

That lead replaces calcium in bone as phosphate rather than by uniting directly with the protein of the organic matrix has been demonstrated. When the organic material in bone particles is destroyed by ignition, lead is much more readily taken from solution than when the calcium phosphate of the bone is removed by decalcification. There is a distinct difference between the removal

TABLE 7

Rate of absorption of lead

NUMBER	INITIAL AMOUNT OF LEAD IN SOLUTION	FINAL AMOUNT OF LEAD IN SOLUTION	TIME OF ABSORPTION
	mgm.	*mgm.*	*min.*
1	25	15.89	¼
2	25	10.78	1
3	25	6.64	2
4	25	4.44	3
5	25	1.49	6
6	25	0.30	9
7	25	0.12	12
8	25	0.00	15
9	25	0.00	30

TABLE 8

Replacement of calcium in bone by lead

NUMBER	INITIAL MOLAR CONCENTRATION Pb × 1000	MOLS OF Pb ABSORBED × 1000	MOLS OF CALCIUM LIBERATED × 1000	$\frac{\text{MOLS OF Pb ABSORBED}}{\text{MOLS OF Ca LIBERATED}}$
1	1.38	1.36	1.09	1.25
2	2.07	2.05	1.82	1.12
3	2.78	2.66	2.08	1.28
4	3.45	3.22	2.28	1.41
5	4.14	3.80	2.41	1.57
6	5.52	4.36	2.74	1.59

pH = 7.4. Volume = 175 cc.

of lead by crushed and ignited bone on the one hand, and by decalcified bone and kelp charcoal on the other. The two latter apparently *adsorb* the lead while the first two replace calcium chemically by lead. The microscopic appearance of the absorbed lead is different in the two cases. Sections show that lead is deposited as a dense, compact layer on the surface of ignited or normal bone; while in decalcified bone it penetrates deeply. The variations in degree

of absorption of lead by these four substances are recorded in table 10 and figure 12.

The absorption of lead by cartilage. As noted above, cartilage has been found to take calcium from solution, and recent experiments

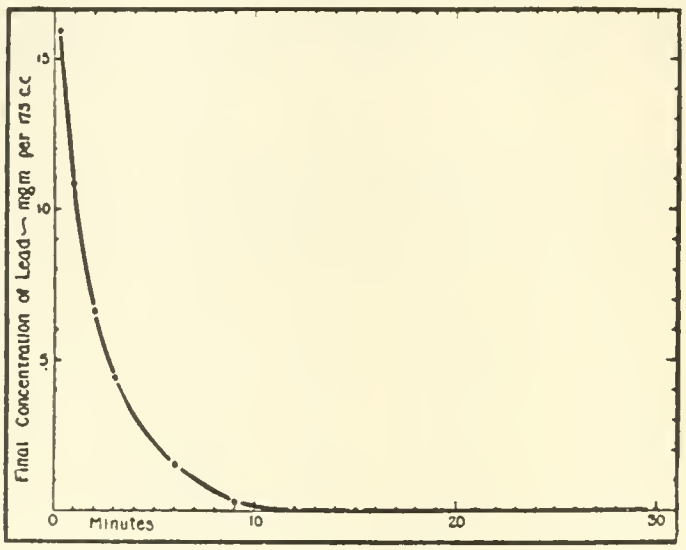


FIG. 9. RATE OF ABSORPTION OF LEAD BY BONE

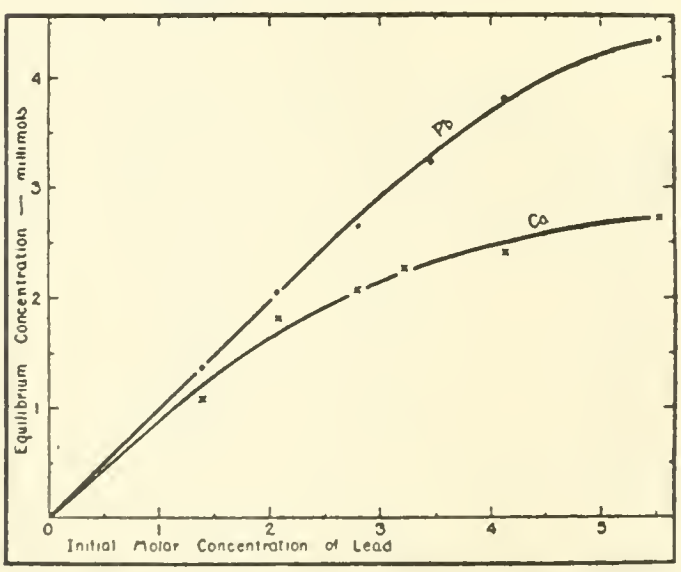


FIG. 10. CHEMICAL REPLACEMENT OF CALCIUM IN BONE BY LEAD

have also demonstrated its power to remove lead from the surrounding medium. The process, unlike that of the absorption of lead by bone, does not bind the lead closely and is not complete. It may possibly be adsorption rather than chemical union. The quantity of lead absorbed is directly proportional to the time of contact and

varies with the hydrogen ion concentration of the solution. Impregnated cartilage gives up its lead much more readily in acid solution than does crushed bone after the same treatment. If the lead combined directly with the cartilaginous protein, as it evidently does, this would be expected, because lead albuminate is very soluble

TABLE 9

Effect of hydrogen ion concentration upon absorption of lead

NUMBER	INITIAL AMOUNT OF LEAD IN SOLUTION	FINAL AMOUNT OF LEAD IN SOLUTION	pH
	mgm.	*mgm.*	
1	125	72.3	4.0
2	125	45.4	5.0
3	125	34.8	6.0
4	125	16.8	6.8
5	125	12.3	7.2
6	125	10.8	7.4
7	125	7.8	7.6
8	125	6.5	7.8
9	125	15.1	8.0

TABLE 10

Absorption of lead by crushed, decalcified and ignited bone, and kelp charcoal

NUMBER	Pb IN SOLUTION	LEAD IN SOLUTION			
		Crushed bone	Decalcified bone	Ignited bone	Kelp charcoal
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	25	0.00	3.72	0.00	4.77
2	50	0.40	10.09	0.00	20.45
3	75	0.89	27.64	0.00	40.12
4	100	7.23	45.48	0.77	56.06
5	125	8.05		2.96	
6	150	17.50	89.99	11.03	90.71
7	200	42.00		19.42	

in an acid medium—it dissolves at hydrogen ion concentrations slightly above pH 5.8.

These experiments with crushed bone do not demonstrate that lead is taken from the circulating blood stream *in vivo* by direct chemical replacement; but they do both explain the removal of lead by bones, either dried or freshly dissected from the animal, and emphasize the sensitiveness of absorbed lead to changes in hydrogen

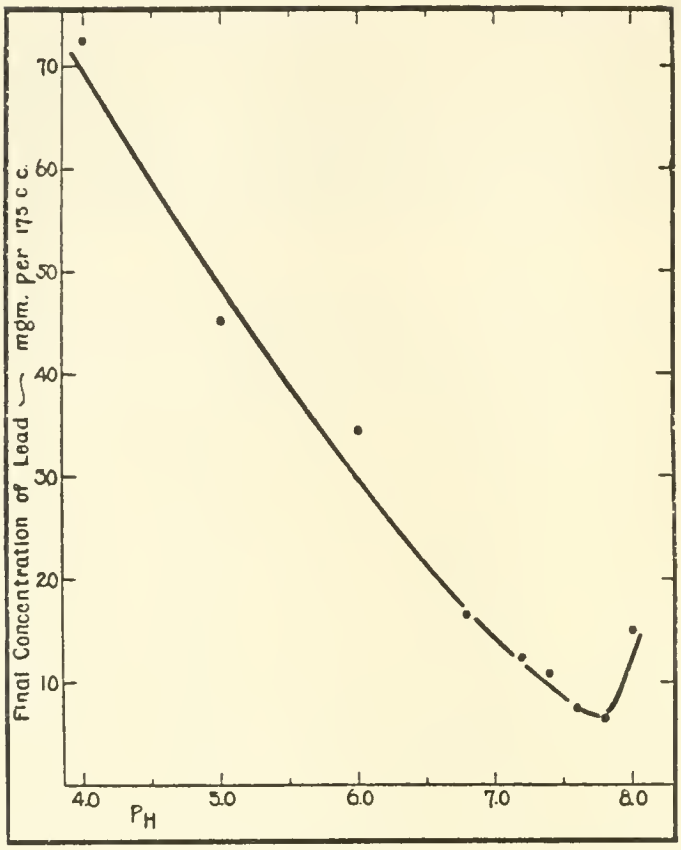


FIG. 11. EFFECT OF HYDROGEN ION CONCENTRATION UPON ABSORPTION OF LEAD BY BONE

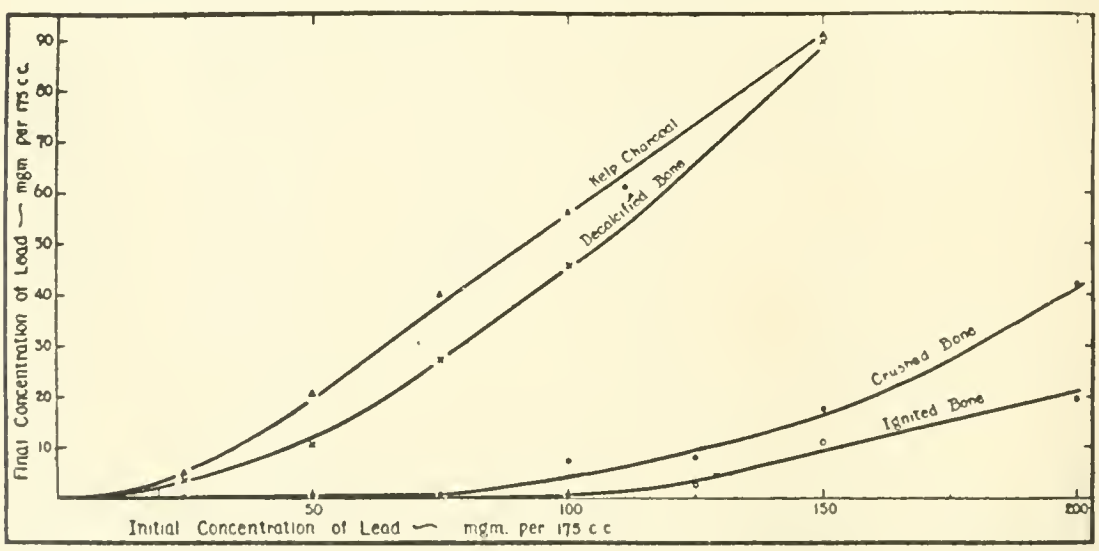


FIG. 12. ABSORPTION OF LEAD BY CRUSHED, DECALCIFIED, AND IGNITED BONE, AND BY KELP CHARCOAL

ion concentration. *In vitro* the deposition of lead is comparatively gross as compared with that in the bones of an animal slowly being poisoned with lead.

Although the ratio of calcium to lead in bone during life must be very much higher than during experiments *in vitro* our results probably indicate the general direction of the reactions.

The data indicate that lead is slowly deposited in bone—possibly by adsorption of colloidal tri-lead phosphate. When deposited this is very inert and insoluble under normal conditions; but any changes in the reaction of the tissues, either toward the acid or alkaline side, allow it to be gradually transformed into the di-lead salt which is one hundred times more soluble.

Local changes of the reaction of tissues may therefore mobilize the lead within the organism—particularly if lead is deposited near the blood vessels of the bones where it can be affected by any variations in the reaction of the blood.

Summary. Lead is probably transported in the blood stream in the form of colloidal lead phosphate and deposited in the bone tissue as tertiary lead phosphate. This is sensitive to changes in acidity, particularly to variations caused by such acids as lactic. This may in part account for the fact that in the body the concentration of lead in muscle is very low and that a majority of the deposited lead is in bone tissue. The sensitiveness of tertiary lead phosphate to changes in hydrogen ion concentration may be an important factor in the frequent development of acute lead intoxication following acute infection or acidosis.

V. LEAD ABSORPTION

Information regarding the rates and methods of absorption of lead by various routes is of great importance both from a physiological and practical point of view. Industrially the main portals by which lead enters the body are the respiratory tract, the gastro-intestinal tract, and the skin. The absorption of lead introduced into the subcutaneous tissue and the intraperitoneal cavity is of experimental interest.

Since investigation of the problem of lead absorption was first stimulated by industrial tragedies, it is not strange that cutaneous absorption was given an important place by many early observers. Even today surely the most obvious exposure of workmen to lead is by way of the skin. The characteristically besmeared hands and

faces of painters and the covering of dust on the clothes and features of men handling dry lead compounds repeatedly raises the question: Can lead be absorbed by the intact skin?

Manouvrier (280) in a review of the literature up to 1873 quotes among others Canuet (66) (1825), Brambilla (53) (1837), Borghi (46) (1840), Bricheteau (54) (1848), and Fievée de Jeumont (136) (1855) as supporters of the view that lead may be absorbed through the skin. To Manouvrier (280), Eichhorst (110), Malherbe (276), Rousseau (392), and others further evidence of local absorption through the skin has seemed to be furnished by the frequent appearance of paralysis or arthralgia in those parts most exposed to lead. These views have been persistently preserved and handed down in the literature and we find as recent a writer as Oliver (337) suggesting the possibility of cutaneous absorption of lead from cosmetics.

When experimental studies are employed, however, to test the validity of this plausible and attractive theory the results seem on the whole to be negative.

Tanquerel des Planches (453) (1839) was unable to produce symptoms of poisoning in dogs and rabbits by daily rubbing the shaven skin with various lead preparations or by the application of diachylon plasters. Similarly, Süssmann (450) (1918) could produce only very slight absorption (as evidenced by the detection of small amounts of lead in the excreta and tissues) by rubbing and poulticing the shaven skin of cats with lead oleate. Rand (375) has pointed out the possible inaccuracy involved in drawing conclusions about cutaneous absorption in man from data obtained from experiments on non-perspiring animals.

Nevertheless such modern authorities as Legge and Goadby (252), Meillère (293), Oliver (337), and Hamilton (188) all agree that the intact skin is practically negligible as a portal of entry for inorganic lead compounds. There is danger, however, in making too liberal generalizations from this statement. Numerous records of cases of plumbism resulting from the application of lead preparations to ulcers, burned areas, skin eruptions, as vaginal douches, or eye washes, etc. (356) (24) (102) (353) (56) indicate that the broken or irritated surfaces may offer a much more ready path of entrance to lead. Neither can deductions as to the rate of absorption of organic

lead compounds through the skin be drawn from experiments with aqueous solutions of lead salts. Many compounds which dissolve fat, as for example methyl salicylate and nitrobenzol, are known to penetrate the skin readily. The general opinion that there is danger of absorption in handling *organic* lead compounds such as lead tetraethyl is apparently well founded. Furthermore, the absence of cutaneous absorption should not be interpreted as a justification for any relaxation in the regulations for personal sanitation among lead workers. Although the hand itself probably does not absorb lead, it may still be the means of carrying lead compounds to the mouth or nose where ready entrance to the organism is offered.

A strictly chronological discussion of the theories of lead absorption is not possible because advocates of all views may be found at any given time and because absorption rarely takes place exclusively by one route except under experimental conditions. Nevertheless, the common practices of sweetening wine with lead acetate (453), of cooking in lead or pewter dishes (453), of conducting public water supplies through lead pipes, and even of using lead compounds medicinally for certain intestinal disorders, made poisoning from gastro-intestinal absorption a much more common occurrence in earlier times. This gave rise to the generally accepted belief, which has persisted in popular opinion until the present, that the lead which enters the gastro-intestinal tract is chiefly responsible for the symptoms of plumbism. Meillère (293) (1903) holds this view and indeed as recently as 1921 in the Agenda of the International Labor Office of the League of Nations the following statement was made: "The digestive canal is undoubtedly the most important path of entry . . . . the respiratory canals have only a secondary importance in this connection and even this is still contested."

Absorption through the respiratory tract. The fact that lead is absorbed by the gastro-intestinal tract is doubted by no one. Both experimental and accidental cases of lead intoxication have repeatedly occurred when lead could not possibly have entered by any other route. Moreover Carlson and Woelfel (68) have shown that even the most insoluble lead compounds dissolve to some extent in gastric juice. On the other hand, the persistent reiteration from Stockhusen's (444) time to the present, by students of the industrial lead hazard, that the

dusty trades are the most dangerous, suggests that more attention should be given to the respiratory tract as a portal of entry.

As early as 1840 Tanquerel (293) (page 89, vol. i), performed one experiment to test this point. Minium was insufflated through a tracheal cannula in a dog, and fatal lead poisoning gradually developed, and thus proved that lead had been absorbed. Little attention was given to this result, however, and not until 1912 do we find another serious attempt to demonstrate experimentally that lead is absorbed through the respiratory tract. At this time Goadby (165) showed that when animals were confined in an atmosphere of lead dust, toxic symptoms developed more quickly than when ten times as much lead was administered by mouth. Although the possibility of simultaneous gastro-intestinal absorption was not excluded, these experiments clearly indicate that pulmonary absorption is by no means negligible.

Extensive studies of this problem have recently been made (308). In the first series of experiments designed to prove definitely that lead compounds are absorbed by the respiratory tract, the following technique was employed:

Cats were prepared by aseptically ligating the esophagus to render swallowing impossible. Suspensions of finely divided lead carbonate, oxide, chromate, or sulphide in sterile physiological salt solution were then introduced into the trachea as near the bifurcation as possible. After varying lengths of time the animals were killed and the amount of lead present in all the tissues except the respiratory tract was determined.

The fact that even after only a few hours significant amounts of lead (20 to 50 mgm.) were found distributed in the various tissues leaves no doubt about absorption of even the most insoluble lead compounds by the lungs. The objection may, however, be raised that where dust is simply inhaled very much less reaches the lungs than when a suspension is artificially introduced (328) (254) (394). This is probably true, but the work of Blumgart (40) in this laboratory has shown that this factor does not greatly alter the rate of absorption.

He introduced solid lead carbonate particles into the nasopharynx in dogs prepared by means of a tracheal cannula and ligation of the esophagus,

so that lead could enter neither the lungs nor the gastro intestinal tract. After one to three days had elapsed these animals were killed and the quantity of lead present in all the tissues exclusive of the head was determined. Here again evidence of the rapid absorption showed that lead dust needs merely to reach the nasal passages in order to gain ready entrance into the organism.

These somewhat drastic experiments prove conclusively that lead is absorbed by the entire respiratory tract, but the experimental subjects were in too abnormal a condition and the duration of the experiments was too limited to allow a comparison of the course of events in the intoxications following gastro-intestinal and respiratory absorption. Consequently another series of experiments was carried out in which various lead compounds were introduced into the respiratory tract of normal animals. This was accomplished with very little respiratory disturbance by inserting a needle through the unbroken skin into the lumen of the trachea and injecting approximately 2 cc. of a suspension of lead carbonate or other salt in physiological saline. Undoubtedly some lead was swallowed in these cases so that there was some gastro-intestinal absorption, but the development of symptoms was so much more rapid in these animals than in the cats which had received far greater quantities of soluble lead by mouth that the relative danger involved in the two forms of exposure was clearly demonstrated. Lead lines appeared within four or five days and such typical signs of plumbism as loss of appetite, constipation, weakness and nervous symptoms developed rapidly. If the insufflation of dust was repeated, intoxication soon became fatal.

A comparison of the quantities of lead absorbed into the organism by different routes is given in table 11. The fact that during long experiments the disproportion between the quantities of lead retained after absorption from the respiratory and gastro-intestinal tracts decreases, it makes evident that, if time allows, excretion becomes very important. When lead enters the body by way of the respiratory tract, both absorption and excretion involve transportation in the systemic blood; while lead entering through the gastro-intestinal tract may be excreted without being absorbed, or after absorption may be caught by the liver and excreted in the bile without ever reaching

the systemic circulation. Thus a large proportion of the lead absorbed from the gastro-intestinal tract is confined to a region where it causes relatively little damage.

The course which lead follows from the time of entrance into the organism until it is excreted or stored explains in part the great danger involved in exposure to lead dust, and makes it quite evident that the dusty trades are the most hazardous because they allow absorption through the respiratory tract. Figures 13 and 14 illustrate diagrammatically the course of lead within the body. The black circles

TABLE 11

Comparative rates of lead absorption by different routes

NASO-PHARYNX (FROM BLUMGART)			LUNGS			GASTRO-INTESTINAL TRACT		
Pb insufflated as $PbCO_3$	Duration of experiment	Pb in body exclusive of head	Pb insufflated as $PbCO_3$	Duration of experiment	Pb in body exclusive of lungs	Pb fed as lead acetate solution.	Duration of experiment	Pb in body exclusive of gastro-intestinal contents.
mgm.	*hours*	*mgm.*	*mgm.*	*hours*	*mgm.*	*mgm.*	*days*	*mgm.*
480	18	8.0	250	19	26.6	2,000	9	5.8
410	24	24.6	250	24	16.7	2,580	18	24.8
180 300	30–36	30.6	250	42	72.4	2,800	30	37.1
300 470	30–36	24.3	250	72	71.9	7,110	67	23.9
			250	90	35.1	11,400	99	86.3
			250	94	42.6	6,280	124	88.9

represent areas of injury, the hollow circles comparatively resistant areas.

Gastro-intestinal absorption. Though the stomach is chiefly responsible for the solution of swallowed lead particles, there is no evidence that the absorption of lead is confined to any special part of the gastro-intestinal tract. Some difference of opinion does, however, exist as to the region where absorption is most rapid.

Meillère (293) and Brouardel (56) believe that any mucous surface—hence the entire alimentary canal—can absorb lead. Legge and Goadby (252) state that absorption is very slight in the stomach and takes place most

rapidly in the upper part of the small intestine. Somewhat opposed to this view is the opinion of Harnack (200) that bile so reduces the rate of absorption that it becomes very slight in the part of the intestine where the bile enters.

The presence of food, and more especially of milk, in the gastro-intestinal tract has been generally supposed to decrease greatly the rate of lead absorption. This theory is based upon the arguments

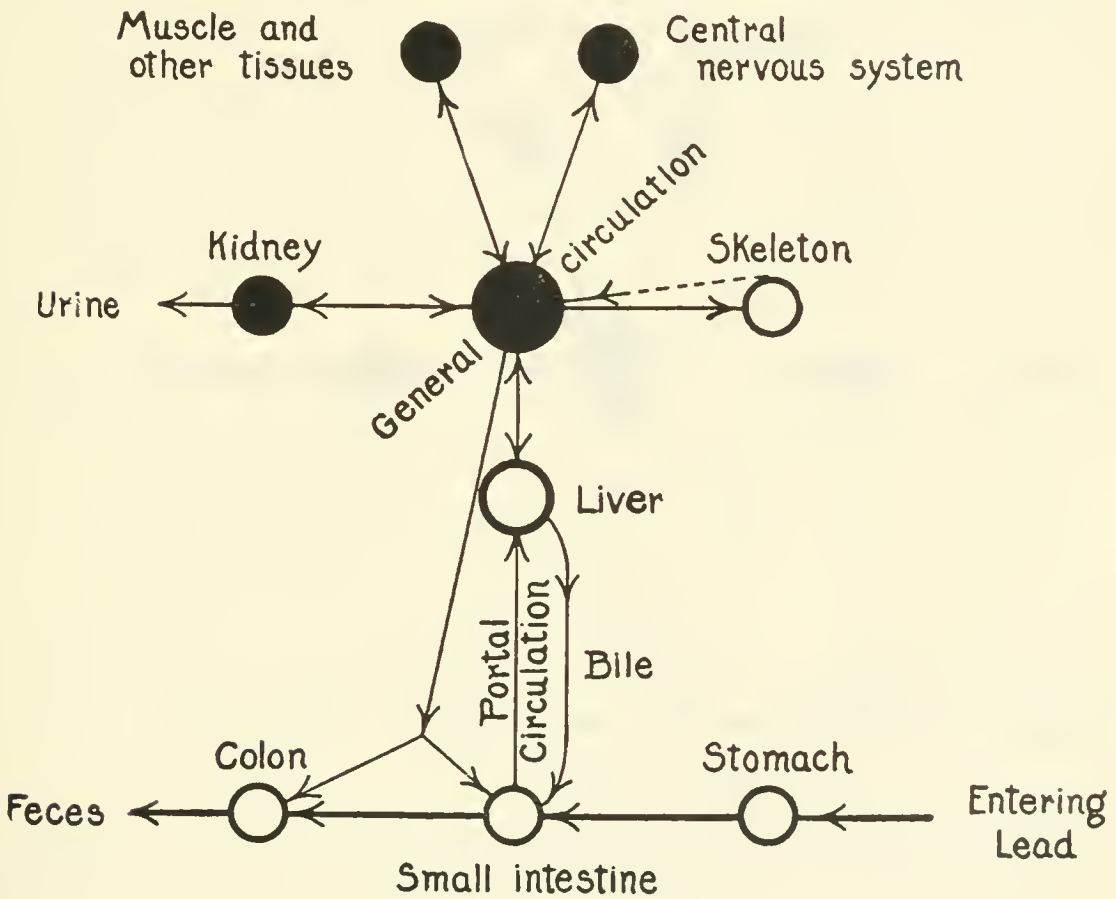


FIG. 13. DIAGRAM ILLUSTRATING THE COURSE OF LEAD WITHIN THE BODY AFTER ENTRANCE FROM THE GASTRO-INTESTINAL TRACT

The solid black circles represent areas susceptible to injury; the hollow circles comparatively resistant areas.

(*a*) that solution of solid lead compounds in the stomach is retarded by the reduction of acidity, and (*b*) that the food itself unites with lead to form comparatively insoluble compounds.

In the course of experimental work in this laboratory, lead acetate solution mixed with 75 cc. of milk was administered to one group of cats while

to others the same doses (50 mgm. of lead per kilo of body weight) of lead were given in water twelve to twenty hours after the last food had been taken. In both cases lead was given on alternate days until death. As the figures in table 12 indicate, there was no consistent nor significant difference between the quantities of lead retained in the body by the two groups of animals.

Thus, although the general belief in the value of a milk diet as a preventive of lead poisoning is undoubtedly well founded as will be

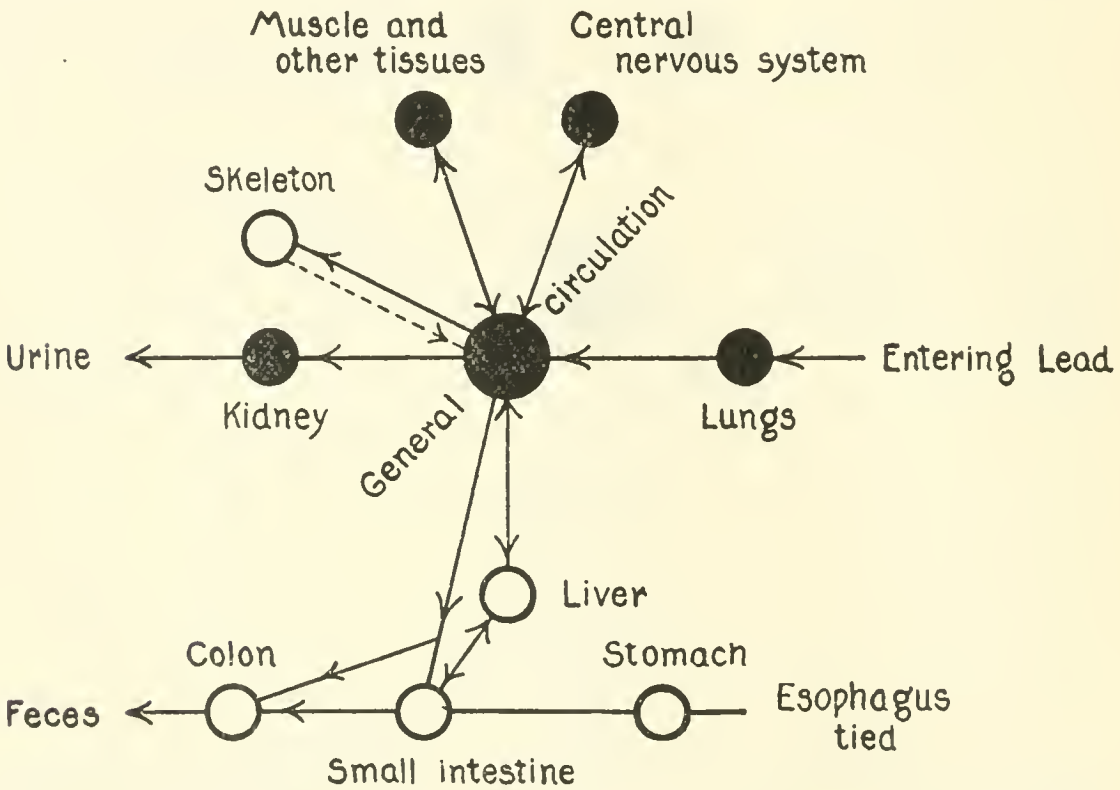


FIG. 14. DIAGRAM ILLUSTRATING THE COURSE OF LEAD WITHIN THE BODY AFTER ENTRANCE FROM THE LUNGS

The solid black circles represent areas susceptible to injury; the hollow circles comparatively resistant areas.

shown later (section IX, page 101); the beneficial effect of milk is due to its dietary properties rather than to any effect on the absorption of lead.

The exact processes involved in absorption of lead from either the respiratory or gastro-intestinal tract are not clearly understood; but certain observations have been made which point to the type of physiological reactions which occur. In the lungs the presence of poly-

morphonuclear leucocytes and mononuclear phagocytic cells containing lead indicates that phagocytosis probably aids absorption. The invariable storage of lead in the skeleton as the phosphate, no matter in what form it was administered, furnishes ample proof of true solution during absorption or transportation. Even with the extremely sensitive test which Cazeneuve (73) devised for chromic acid, no chromate could be found in the bones after insufflation of lead chromate (308). To permit such solution a relatively high degree of acidity is necessary. Obviously, ingested lead can be dissolved in the acid of the gastro-intestinal tract (68), but in the lungs some complicated mechanism must be involved in producing sufficient acidity. Perhaps the ready penetration of CO_2 through cell membranes (226) (227) permits the hydrogen ion concentration of the cells to increase to the point where solution can take place.

TABLE 12

Effect of milk on the rate of gastro-intestinal absorption of lead

WITH MILK				WITHOUT MILK			
Number of cat	Duration of experiment	Total lead administered	Total lead found in tissues	Number of cat	Duration of experiment	Total lead administered	Total lead found in tissues
	days	*grams*	*mgm.*		*days*	*grams*	*mgm.*
10	43	3.78	16.00	148	18	2.58	24.85
381	55	3.74	46.48	12	30	2.70	38.07
179	95	4.72	23.00	7	66	7.14	28.89
144	99	11.40	86.33	149	124	6.28	88.99

Subcutaneous absorption. Subcutaneous injections have frequently been used as an experimental means of inducing very gradual absorption of lead. Straub (448) and Erlenmeyer (122) seem to have been most successful with this method and have frequently produced in cats a fatal and very chronic type of plumbism. In this laboratory the subcutaneous injection of 220 mgm. of lead as carbonate failed to produce any toxic effects except the appearance of the lead line in a few cases. Several times, however, local necrosis resulted. On the whole, the method was quite unsatisfactory in our hands. Some absorption of lead from the subcutaneous deposit did, however, occur for several weeks after the injection, for the body tissues, exclusive of the immediate region of the deposit, were found to contain

lead (see table 13). The lead absorbed is of course represented not only by the amount found in the tissues but also by the quantity excreted. This is indicated by the discrepancy between the amount injected (220 mgm.) and the amount recovered. Lead absorbed from such a deposit, like that from the lungs, enters directly into the general circulation, but in these cases so gradually that the animals remain in good condition and toxic symptoms develop very slowly if at all, despite a definite absorption of lead.

A point of practical interest in this connection is the possibility of lead absorption from bullets embedded in the tissues. The literature on this subject is rather contradictory.

TABLE 13

Analysis of tissues of cats after lead absorption from subcutaneous tissue

NUMBER	DURATION OF EXPERIMENT	AMOUNT OF LEAD INJECTED	UNABSORBED LEAD IN DEPOSIT	LEAD IN TISSUES
	days	*mgm.*	*mgm.*	*mgm.*
1	132	220	110.45	25.72
2	149	200	157.99	29.20

Lewin (262), Leoper and Verpy (269), and Disselhorst and Schneider (99), and Oliver (339) may be mentioned among those who have reported toxic symptoms produced by the lead from bullets. Bonhoff (43), however, who has done the most recent and extensive work on the subject, concludes that absorption of lead from embedded bullets is very rare.

Summary. The route by which lead enters the organism most rapidly is the respiratory tract. Hence, the exposure to be most guarded against is the inhalation of finely divided particles. Next in danger comes the continued ingestion of lead compounds, and last of all the possible slow cutaneous absorption in persons with tender, irritated, or broken skin.

VI. DISTRIBUTION AND STORAGE OF LEAD

Storage of lead in the organism is suggested by the characteristic latency and frequent recurrence of chronic plumbism. Ever since analytical methods have been available, accurate chemical information about the mechanism of these processes has been sought. As

a result, the literature since 1840 contains a tremendous amount of data on the lead content of the tissues and excreta of men and animals during plumbism. But because most of the investigations include examinations of only one or two tissues most directly involved in the clinical symptoms, a review of these data adds nothing to a real understanding of the fate of lead in the organism. The few more extensive studies deserve recapitulation because they provide an outline of what was known about the distribution and storage of lead before this investigation.

In 1861, Gusserow (182) published a most suggestive paper which should have influenced later work more than it apparently has. By means of a somewhat crude electrolytic method he analyzed the various tissues of poisoned rabbits, and found by far the highest concentration of lead in the bones. Muscles, kidney, liver, and central nervous system came next in order. In discussing his observations Gusserow suggested an analogy between the storage of lead and calcium in the skeleton and hinted that changes in the organism as a whole might give rise to migrations of lead as well as of calcium to and from the bony deposit. This suggestive idea was ignored by Gusserow's contemporaries, but much attention was focused upon his statement that the muscles contain a relatively higher concentration of lead than do the other soft tissues. On the basis of this work and without further experimentation, Rosenstein (390), Hitzig (218), and others soon proceeded to explain the entire syndrome of chronic plumbism in terms of lesions produced by lead on muscles. Heubel (213), who made an extensive chemical study of the distribution of lead among the tissues by means of the gravimetric sulphate method, was opposed to this view. Like Gusserow, he found the greatest concentration of lead in the bones; but observed that the central nervous system contained much more than did muscle. He argued that because of its chemical affinity for lead and its susceptibility to toxic effects, the nervous system must suffer lesions which are at least partly responsible for the symptoms of lead poisoning. Similar conclusions were reached by Ellenberger and Hofmeister (113) in their study of sheep poisoned by lead.

A very thorough and critical study of lead poisoning was carried out by Prévost and Binet (368) in 1889. Their quantitative data were limited by the fact that in many cases rats and guinea pigs were used as experimental subjects. The individual tissues of these small animals contained too minute amounts of lead to be measured accurately by the gravimetric

chromate method of analysis which these authors employed. They did, however, point out the differences in distribution of lead during acute and chronic plumbism and showed that in acute cases of poisoning following gastro-intestinal absorption the liver contains the largest quantity of lead, with kidney and bone next in order of concentration. In more chronic cases they observed that the concentration of lead in the skeleton is much higher than that in any other tissue. They believed that the lead exists in the bones as the phosphate and that it can be retained in this form indefinitely.

In 1903 Meillère (293) published a large mass of data on the amount of lead in human tissues during lead poisoning. Unfortunately he merely reports the limits of concentrations found in the various tissues in all his collected cases, and thus renders a comparison of individual pictures impossible. Nevertheless, in his discussion, Meillère emphasizes the differences in distribution at various stages of the disease and reports that in old chronic cases there is a progressive selective localization of lead in the bones and keratinous tissue. This he considers is brought about by a kind of internal excretion of the lead to a harmless deposit. The possibility of its mobilization is not discussed in his work.

The innocuousness of the comparatively large amounts of stored lead as contrasted with the marked toxicity of smaller quantities of lead in the process of absorption has been somewhat explained by the work of Straub (447) (448) and Erlenmeyer (121) (122). They poisoned animals fatally by subcutaneous administration of lead carbonate and by means of the gravimetric sulphate method determined the lead present in the animals and excreta. They found that comparatively little lead was retained in the tissues. Much of the injected lead was, however, absorbed and recovered from the excreta. During transportation, toxic symptoms developed which proved fatal in many cases. From these data Straub and Erlenmeyer concluded that the effects of lead are produced not by lead deposited at the site of injury, but rather by the "lead stream"—that is, by the small amount of soluble lead actually carried in the blood. They argued that while the amount of lead comprising this "stream" is too small to be effective in a single dose, the influence of innumerable subminimal doses could eventually cause pathological changes in certain sensitive tissues.

An attractive theory to explain the latency and recurrence of chronic plumbism could be constructed by assembling the suggestion of Gusserow, the chemical data furnished by Heubel, Prévost and Binet, Meillère and others, and these suggestions of Straub and

Erlenmeyer. It is possible that mobilization of the lead accumulated and stored in the bones might produce a "lead stream" of sufficient concentration to evoke toxic symptoms. But since these various investigations were carried out by such different and often inaccurate methods and included such varied types of lead poisoning, the proper place of each work in completing the picture of the fate of lead in the organism can be ascertained only after the problem has been studied by an accurate and uniform method. This has recently been done in this laboratory (307) (308) (309).

"Normal lead." Before an evaluation can be made of the significance of the quantities of lead found in various tissues, it is necessary to determine whether lead is commonly present in the body. As Chevreul (80) and Devergie (96) long ago pointed out, the term "normal lead" is poorly chosen. Lead is not an essential constituent of any human tissue but it may be present accidentally if the customary activities and diet of an individual allow it to enter the organism. There is only a quantitative difference between so-called "normal lead" and that found in cases of lead poisoning. It is, therefore, important to determine how much lead is likely to be absorbed by individuals living a usual normal life at a given time and place. The following figures for natural lead, published in 1840 by Devergie, although probably inaccurate because of the methods of analysis, have historical interest.

SOURCE OF TISSUE	LEAD FOUND AS $PbSO_4$ IN TOTAL ORGAN
	gram
A new born infant, intestinal tube	0.001
Child eight years old, stomach	0.004
Child four years old, intestinal tube	0.025
Adult healthy woman, stomach	0.020
Adult healthy woman, intestines	0.040
Adult man, intestines	0.031
Adult man, gall bladder and bile	0.003
Sick woman, intestines	0.010
Sick woman, brain (1 pound)	0.006

At about the same time Legrip (253) and Millon (305a) found lead generally present in the blood and tissues of normal individuals.

The practice of sweetening wine with lead acetate, the common employment of pewter dishes and the indiscriminate use of lead pipes, and the medicinal use of lead compounds for certain intestinal disorders can readily explain the figures obtained at this early date. As more care was gradually exerted to obtain pure food and drinking water, the amount of "normal lead" would be expected to decrease. Meillère's (294) figures indicate that this occurs, for in 1903 he found small traces of lead occasionally, but by no means universally, in the tissues of individuals who were not industrially exposed to lead and showed no symptoms of plumbism. This was most frequently found in the hair, nails and bones where it appeared to be stored as a harmless deposit.

Since previous investigations as well as recent work in this laboratory have demonstrated that the lead retained by an apparently normal individual is held almost exclusively by the skeleton, it has seemed reasonable to test specimens of bone for "normal" lead. Table 14 shows the concentrations found in twenty-six specimens of human bone obtained from patients with no symptoms of plumbism and no recent industrial exposure to lead. In seven cases lead was detected. Of this number four patients were found to have had a definite history of exposure at some previous time; in one case this lead had been retained at least nine years without producing any toxic symptoms. The other three gave no history of known exposure to lead but possible exposure might readily have been overlooked. Nineteen of the bones analyzed contained no lead. Most of these were taken from old persons who had had more opportunities for exposure than would younger individuals. Our figures indicate that in this vicinity foods, public water supplies which largely run through lead pipe, and the usual activities of daily life cause no retention of lead in the body. (See Addendum.)

Distribution of lead in experimental lead poisoning. Any study of the distribution of lead in the body must include only cases with accurately known histories of exposure to lead and must be based upon the analyses of all the body tissues from different types and at different stages of the disease if it is to do more than contribute to the existing confusion. Data thus obtained would serve as a basis for the interpretation of some of the figures already reported

in the literature, as well as for the explanation of cases in which less complete chemical studies are possible. To obtain information of this kind, the use of experimental animals is, of course, necessary.

After some preliminary trials with other species in this laboratory, cats were found to be the most satisfactory subjects for such an investigation. Although readily poisoned by lead introduced through any path of entry, they are sufficiently resistant to the intoxication to allow the development of typical chronic plumbism. They are, also of such a size that the entire body may be completely analyzed,

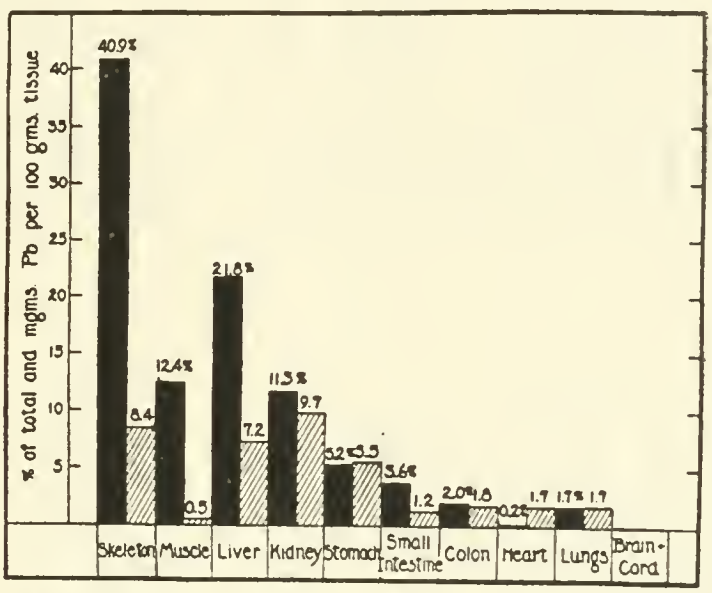


FIG. 15. DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN THE TISSUES OF CAT D

See table 15. Solid black portions represent percentages of total lead in body. Cross-hatched portions represent milligrams of lead per 100 grams of fresh tissue.

and consequently both the percentage of total lead retained and the concentration per unit weight may be determined for each tissue. A few experiments, however, carried out with dogs, rabbits, and hens show the same distribution of lead as reported here for cats.

Effect of the portal of entry on distribution of lead. The contradictory figures for the distribution of lead which are reported constantly by different investigators, suggest the possibility of some relationship between the portal of entry and the localization of lead in the body. Harnack (200) emphasized this point some time ago and offered as an explanation for the different findings reported by

TABLE 14

Analysis of normal human bones

NUMBER	SOURCE OF SPECIMEN	WEIGHT OF SPECIMEN	LEAD FOUND	LEAD PER 100 GRAMS	SEX	AGE	BRIEF HISTORY
		grams.	*mgm.*	*mgm.*			
1	Femur	152	0.00	0.00	Male	60	Tinsmith for twenty-five years. Leg amputated for diabetic gangrene
2	Tibia	210	0.00	0.00	Female	57	Housework until three years before. Amputation of lower leg for arteriosclerotic gangrene
3	Ribs	51	0.00	0.00	Female	27	Clerk. Rib resected for unilateral pulmonary tuberculosis
4	Ribs	75	0.00	0.00	Male	31	Weaver in cotton mill for three years. Resection of ribs for chronic empyema
5	Tibia Fibula Tarsus	332	0.87	0.26	Male	11	Traumatic amputation of leg. Only known exposure to lead was careless use of paint two months before
6	Tibia Fibula Toe	225	0.00	0.00	Male	63	Weaver. Amputation for arteriosclerotic gangrene
7	Lower leg	350	0.00	0.00	Male	58	Fireman. Amputation for osteomyelitis
8	Vertebra	60	0.00	0.00	Male	44	Occupation unknown. Lobar pneumonia
9	Tibia Fibula	225	0.00	0.00	Male	15	Stock clerk for three months. Amputation for sarcoma
10	Vertebra	63	0.74	1.18	Male	60	Veterinary. Until fifteen years before death had used drinking water from well contaminated with lead
11	Tibia	351	0.00	0.00	Male	69	Night watchman for six months. Usual occupation unknown. Amputation of left leg for arteriosclerotic gangrene
12	Tibia	208	0.00	0.00	Male	50	Tailor. Amputation of a painful stump for osteitis

13	Tibia	162	0.00	0.00	Female	66	Housewife. Amputation of unhealed stump for diabetic gangrene
14	Foot	245	0.22	0.09	Male	38	Brakeman on railroad for twelve years, except for occasional painting. Painter seven years previous to present occupation. Traumatic amputation
15	Tibia	362	0.00	0.00	Male	75	Occupation unknown
16	Vertebra	61	0.72	1.18	Female	58	Demonstrator and saleswoman. Frequently painted pictures, furniture, and interior of her house. Syphilis of stomach and cirrhosis of liver
17	Tibia Fibula Foot	810	0.00	0.00	Male	62	Mill worker. Amputation for arteriosclerotic gangrene
18	Tibia	205	0.00	0.00	Male	52	Occupation unknown. Traumatic amputation
19	Femur	420	0.00	0.00	Male	58	Coal heaver for twenty years. Amputation
20	Radius and ulna	245	0.00	0.00	Male	45	Laborer. Traumatic amputation of forearm
21	Femur	235	0.00	0.00	Male	15	School boy. Amputation for septicemia
22	Tibia	340	7.08	2.08	Male	60	Treer in shoe factory for thirty years. No known exposure to lead. Amputation for diabetic gangrene
23	Femur	300	0.00	0.00	Male	64	Mechanic for fifteen years. Amputation for arteriosclerotic gangrene
24	Femur	217	0.00	0.00	Male	33	Twelve years in rubber plant, spreader and curer of rubbers. Amputation for progressive thromboangiitis
25	Tibia	132	1.21	0.90	Female	67	Housewife. No known exposure to lead. Diabetic gangrene
26	Tibia	335	5.62	1.68	Female	65	Cook. Amputation for diabetic gangrene. Only known exposure to lead was use of lead water pipes for many years

Annuschat (9) and Lehmann (257) the fact that Annuschat had fed lead by mouth while Lehmann had introduced it into the general circulation by means of a subcutaneous injection.

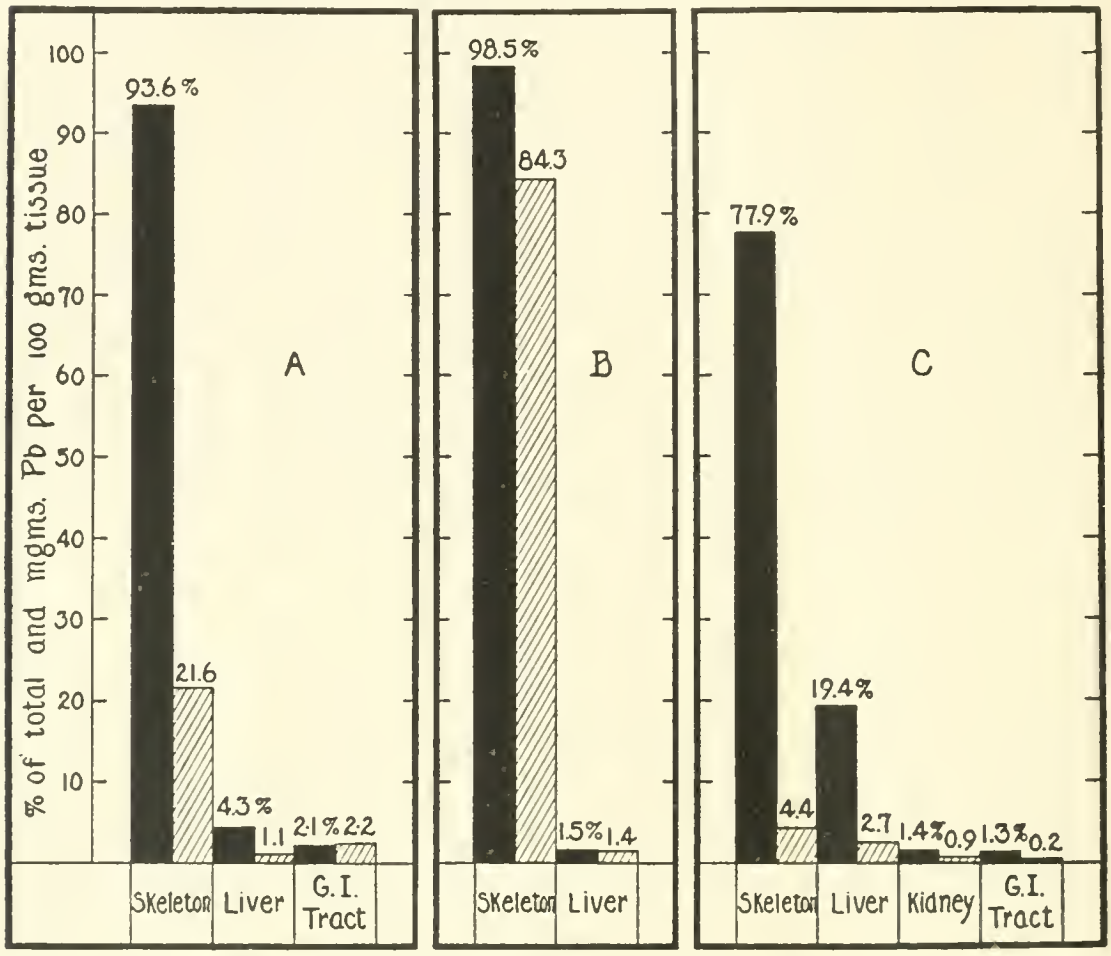


FIG. 16. DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN CATS 2, 5 AND 13 (TABLE 16)

Solid black portions represent percentages of total lead in body exclusive of lungs. Cross-hatched portions represent milligrams of lead per 100 grams of fresh tissue. No lead was determined in tissues not represented in the figure.

A, Cat 2, nineteen hours after insufflation of 5 cc. of lead carbonate suspension. Total lead in body exclusive of lungs, 26.62 mgm.

B, Cat 5, forty-two hours after insufflation of 4 cc. of lead carbonate suspension. Total lead in body exclusive of lungs, 72.40 mgm.

C, Cat 13, ninety-six hours after insufflation of 2 cc. of lead carbonate suspension. Total lead in body exclusive of lungs, 12.71 mgm.

A comparison of the various results obtained in this laboratory during a study of the distribution of lead in the tissues of animals which had received lead by different routes up to the time of death,

throws more light on this problem (307) (308). For ease of comparison the experiments may be classified as follows:

Group A. Absorption by gastro-intestinal tract. The cats in this group were poisoned by a solution of lead acetate administered by stomach tube in doses of 50 mgm. of lead per kilo of body weight every other day.

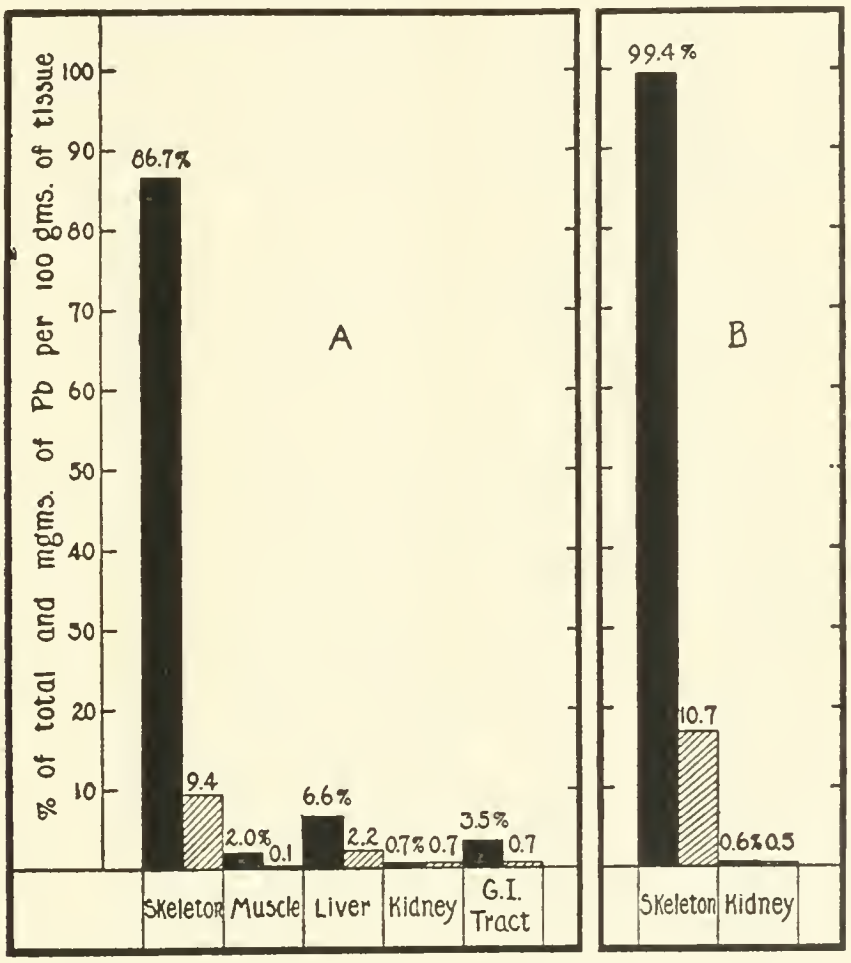


FIG. 17. DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN CATS A AND B FOLLOWING THE SUBCUTANEOUS ABSORPTION OF LEAD

See table 17. Cat A: killed 132 days after the injection of 220 mgm. of lead. Total lead in tissues 25.72 mgm.

Cat B: killed 149 days after the injection of 220 mgm. of lead. Total lead in tissues 29.20 mgm.

Group B. Absorption by respiratory tract. For these experiments the animals were prepared by ligating the esophagus in an aseptic operation. While the animal was still anesthetized approximately 200 to 250 mgm. of lead were introduced into the trachea as a suspension of finely divided lead carbonate in physiological salt solution. In some cases this insufflation

TABLE 15

Collected distribution figures for ten animals poisoned by gastro-intestinal route

ANIMAL	WEIGHT		LENGTH OF EXPERIMENT	TOTAL LEAD		PERCENTAGE DISTRIBUTION OF ABSORBED LEAD IN TISSUES											
	Start	End		Given	In body	Skeleton	Muscle	Liver	Kidney	Stomach	Small intestine	Colon	Heart	Lungs	Brain and cord	Spleen	Omentum
	kgm.	*kgm.*	*days*	*grams*	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Rabbit A	3.6	3.4	9	1.09	5.85	47.3	0.0	29.7	6.0	5.1	4.0	5.5	1.4	1.4	0.0		
Cat C	3.0	2.2	12	1.35	12.86	81.5	5.0	6.3	2.1		3.2*			1.9	0.0		
Cat D	4.3	3.4	18	2.58	24.85	40.9	12.4	21.8	11.3	5.2	3.6	2.0	1.2	1.7	0.0	0.0	0.0
Cat E	3.4	1.8	30	2.80	37.08	84.4	10.3	0.6	0.5	1.4	1.3	1.5	0.6	Trace	0.0		
Cat F	3.3	2.4	43	3.78	11.24	69.9	6.4	6.6	1.4	3.9	2.6	2.2	3.8	0.0	3.2		
Cat G	4.6	2.8	55	3.74	46.48	65.8	1.7	7.9	5.4	7.0	1.2	0.4	7.0	1.4	0.4	0.5	0.6
Cat H	4.0	2.4	67	7.11	23.90	39.0	20.5	14.7	13.6	5.0	0.8	3.0	0.8	1.8	0.6		
Cat I	3.7	1.6	95	4.72	23.00	47.2	4.5	20.6	6.1	2.2	6.1	2.5	0.9	1.8	1.2	1.6	
Cat J	5.5	4.5	99	11.40	86.33	77.2	12.8	7.4	0.3	0.2	0.2	0.6	0.3	0.3	0.1		0.6
Cat K	3.4	1.8	124	6.28	88.99	85.5	4.0	3.6	2.4	1.5	0.1	1.6	0.4	0.2	0.2		

* Gastro-intestinal tract as a whole.

was accomplished by means of a syringe and a small tube introduced through the mouth and nearly to the bifurcation of the trachea, and in others through a needle inserted in the wall of the exposed trachea. No attempt was made to feed the animals but fluid was introduced daily by intraperitoneal injections of physiological salt solution. That absorption continued until death was demonstrated by the presence of unabsorbed lead carbonate in the lungs at autopsy.

Group C. Subcutaneous absorption. In this series of experiments approximately 220 mgm. of lead as carbonate was introduced under the skin of the back. The remainder of this injection which was unabsorbed after death provided evidence that absorption had been continuous until the animals were killed.

Figures 15, 16 and 17 show diagrammatically the distribution of lead typical for Groups A, B, and C, respectively. The analytical data for several animals of each type are presented in tables 15, 16 and 17.

Superficially, the points of similarity in these pictures are (*a*) the high percentage of the total retained lead held in the skeleton, and (*b*) the relatively uniform order of concentration of lead in the soft tissues of the different groups. (Traces of lead could as a rule be detected by the microchemical test in the tissues not shown in the diagram of the animals of Groups B and C.) The most obvious differences, on the other hand, are (*a*) the markedly higher concentrations of lead in the liver and washed gastro-intestinal tract of the animals of Group A, and (*b*) the larger amounts of lead in the tissues of Group B after an absorption period of *hours* as contrasted with the quantities retained by Groups A and C after *days* or *weeks* of absorption.

From a closer study it becomes apparent that in every case, disregarding for the moment the lead in the liver, the same disposal is made of lead which reaches the general circulation. The most striking feature of this distribution appears at once to be the great affinity between lead and bone. The other tissues, in contrast, retain only such small amounts of lead as might be found in any tissue receiving a supply of blood in which lead was transported. In general the relative concentration of lead in the soft tissues is in direct ratio to their vascularity.

TABLE 16

Distribution of lead in cats which have received solid lead carbonate by lung, the gastro-intestinal tract being excluded as a portal of entry

NUMBER	WEIGHT OF CAT	DURATION OF EXPERIMENT	AMOUNT OF SUSPENSION INSUFFLATED	TOTAL LEAD IN BODY*	PERCENTAGE DISTRIBUTION OF TOTAL LEAD				REMARKS
					Skeleton.	Liver	Gastro intestinal tract	Kidney	
	kgm.	*hours*	*cc.*	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*	
1	1.5	12	5.0	2.62	86.6	13.4	Trace	0.0	Poor condition. Bronchopneumonia
2	3.5	19	5.0	26.62	93.6	4.3	2.1	0.0	Fair condition. Some congestion of lungs. 150 cc. 0.9 per cent NaCl solution given intraperitoneally
3	3.2	24	5.0	16.72	90.5	6.0	3.5	0.0	Very good condition. 150 cc. Ringer solution intraperitoneally
4	1.6	24	5.0	18.43	100.0	0.0	0.0	0.0	Poor condition. Much respiratory disturbance. No fluid given
5	3.6	42	4.0	72.40	98.5	1.5	0.0	0.0	Developed bronchopneumonia. No fluid given
6	3.5	48	5.0	16.22	96.1	3.9	0.0	0.0	Many hemorrhagic areas in lungs. 100 cc. Ringer solution intraperitoneally
7	2.0	56	5.0	14.75	92.0	8.0	0.0	0.0	Lungs in fair condition. 75 cc. Ringer solution and 100 cc. 5 per cent glucose solution intraperitoneally
8	3.0	72	5.0	71.87	98.3	1.7	0.0	0.0	Lungs in good condition. 640 cc. Ringer solution intraperitoneally
9	2.1	80	5.0	14.97	69.2	24.7	6.0	0.0	Developed distemper. Right lung pneumonic. Left lung contained lead deposit. Otherwise normal
10	3.0	90	5.0	28.95	80.6	19.4	0.0	0.0	Right lung showed marked congestion. Left lung fairly normal. 450 cc. Ringer solution intraperitoneally
11	3.1	90	5.0	35.08	70.1	24.2	5.7	0.0	Lungs fairly normal. 150 cc. Ringer solution intraperitoneally
12	3.3	94	5.0	42.64	89.0	10.3	0.7	0.0	Pneumonia developed on last day. 550 cc. Ringer solution intraperitoneally

13	2.8	96	2.0	12.71	77.9	19.4	1.4	1.3	Infection at site of wound. Lungs in good condition. 1055 cc. Ringer solution during experiment. Blood CO_2, 20.9 volume per cent at death
14	3.1	120	1.5	26.32	93.9	4.1	1.4	0.8	Lungs in very good condition. 950 cc. Ringer solution during experiment. Blood CO_2, 30.0 volume per cent at death
15	2.5	162	5.0	26.25	87.2	3.8	9.0	0.0	Congestion of upper lobes of lungs. 940 cc. Ringer solution during experiment. Blood CO_2, 19.2 volume per cent at death

* Exclusive of lead remaining in lungs.

When the liver is included in the picture, however, a marked discrepancy is apparent between the percentages of lead retained by this organ in the different groups of experiments. This can be readily explained by a consideration of the different points at which lead may enter the circulating blood when administered in different ways. Lead absorbed in the gastro-intestinal tract is first carried in the portal blood to the liver, which, as is well known, removes foreign or toxic materials from the blood stream. The high efficiency of the liver in removing lead from the portal blood explains the retention of so much lead in this organ. A large percentage of this absorbed lead is gradually excreted in the bile (see work by Brady, section VII, page 78). This mechanism accounts for both the small amount of lead within the rest of the organism and the late appearance of toxic

TABLE 17

Distribution of lead in cats after lead absorption from subcutaneous tissue

NUMBER	DURATION OF EXPERIMENT	WEIGHT OF ANIMAL		UNABSORBED LEAD	TOTAL LEAD IN BODY	PERCENTAGE DISTRIBUTION OF TOTAL LEAD					
		At start	At death			Skeleton	Muscle	Liver	Kidney	Gastro-intestinal tract	Brain and cord
	days	*kgm*	*kgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A	132	2.7	3.3	110.45	25.72	86.7	2.0	6.6	0.7	3.5	0.0
B	149	3.3	4.0	157.99	29.20	99.4	0.0	0.0	0.6	0.0	0.0

symptoms following gastro-intestinal absorption. A small quantity of lead does, however, escape retention by the liver and become distributed throughout the organism by the general circulation.

Lead absorbed from the respiratory tract or from subcutaneous tissue, on the other hand, enters the *systemic* blood directly. Under these conditions the liver receives only the lead carried in the hepatic artery and consequently can retain only an insignificant portion of the total quantity of lead absorbed. Although all the tissues are bathed with lead while it is being transported, the only one to pick up and retain permanently any significant amount is the skeleton.

Effect of time on the distribution of lead. Obviously the next problem to be solved was whether in more chronic cases there is a more marked localization of lead in the bone. Figure 18 which shows diagrammatically the percentage distribution in an animal which re-

ceived lead by mouth for a relatively long time, demonstrates that both the actual amount and the percentage of total lead retained in the skeleton are larger than in shorter experiments. Since the actual quantities of lead in the other tissues change very little as the experiment progresses, they of course become an ever decreasing percentage of the total amount of lead within the body.

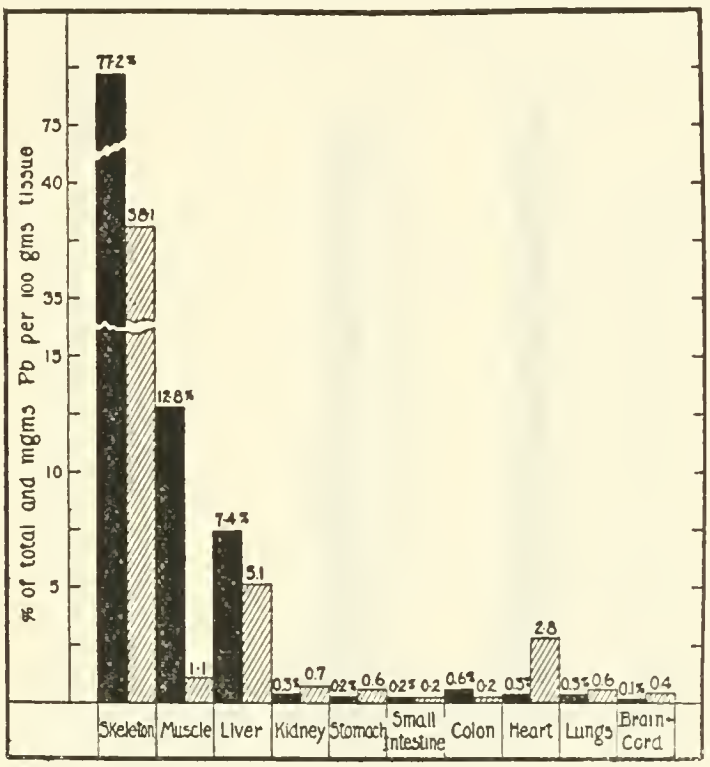


FIG. 18. DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN THE TISSUES OF CAT J

See table 15. After 99 days of lead absorption by gastro-intestinal tract. Solid black portions represent percentages of total lead in body. Cross-hatched portions represent milligrams of lead per 100 grams of fresh tissue.

To test whether lead may be completely localized in the bones after the cessation of absorption, cats were severely poisoned by lead administered by mouth. Just before death seemed imminent the feeding was discontinued and the animals were fed on a normal diet until they gradually recovered. Several weeks after the last dose of lead, when the animals were apparently in perfect health, they were killed and their tissues analyzed. The localization of lead in the skeleton was found to be almost complete (fig. 19).

Thus it is apparent that, from the first, the concentration of lead

in the skeleton progressively increases, while the other tissues do not retain the lead which they receive. Hence, after absorption has ceased, significant amounts of lead are to be found only in an apparently harmless deposit in the bones.

Site of lead storage in the skeleton. Association of the storage of large amounts of lead in the skeleton and the frequent occurrence of marked anemia in chronic plumbism at first suggests that the lead

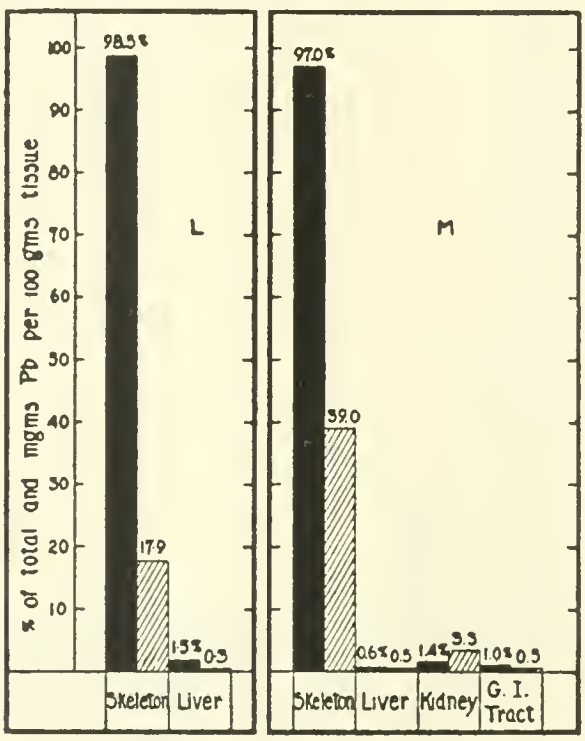


FIG. 19. DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN THE TISSUES OF CATS L AND M KILLED SEVERAL MONTHS AFTER THE LAST DOSE OF LEAD BY MOUTH

Solid black portions represent percentages of total lead in body. Cross-hatched portions represent milligrams of lead per 100 grams of fresh tissue.

Cat L: Killed 82 days after last dose of lead. Total lead in body 20.53 mgm.

Cat M: Killed 160 days after last dose of lead. Total lead in body 97.29 mgm.

by collecting in the bone marrow may interfere with the normal formation of red cells. Animal studies have shown, however, that this does not occur. Separate analyses of the solid shafts of bone and the marrow have shown that the marrow contains only traces of lead comparable to those in the other soft tissues, and the high concentration is to be found only in the solid portion of the bones. Furthermore, the detection of high concentrations of lead in the

pneumatic marrowless bones of the wings of poisoned hens furnishes additional evidence that the marrow plays no essential rôle in the retention of lead by the bones. Prévost and Binet (368) suggested without conclusive experimental proof, and Fairhall and Shaw (129) have recently demonstrated in this laboratory (section IV) that lead exists in the bones as the tertiary phosphate which is extremely insoluble and stable at the ordinary hydrogen ion concentration of the body. Preliminary histological study of the bones after absorption of lead by the organism has demonstrated that lead is usually deposited in the endosteum and occasionally near the capillary walls.

Discussion. This experimental study of the distribution of lead has clarified several points in our general conception of the fate of lead within the organism. When lead is absorbed into the general circulation it is brought into contact with every tissue in the body. There is, however, no marked retention of lead except by the skeleton which paradoxically enough is the tissue apparently least harmed by this toxic agent. In other words, lead is not stored at the site of injury. This fact does not, however, imply that there is an absence of chemical affinity between the soft tissues and lead, but rather that lead compounds which are formed by interaction with these tissues are not retained. The lead, therefore, encounters the various tissues, reacts with them if the proper physiological conditions exist, causing more or less damage, and is then not retained but eventually removed and carried on in the blood stream. This course of events may conceivably be repeated until the lead reaches either some site of excretion or the skeleton. Under normal conditions, a very insoluble lead compound—the tertiary phosphate—is formed in the bony tissue. Since this salt is retained as a skeletal deposit, the bones gradually remove lead from the circulating blood. The two processes of storage and excretion which take place during the absorption of lead continue after absorption has ceased until practically all the lead in the body is held by the skeleton. While this state of affairs exists, the stored lead is apparently harmless, symptoms of plumbism disappear, and the subjects present no evidence whatever of the presence of lead within their tissues. At this point, however, the old theory of Gusserow (182) comes to mind with the disquieting

suggestion that this desirable condition may not be permanent. He advanced no proof for this but recent chemical evidence (sections IV and IX) substantiates the possibility that the skeletal deposit may be released, for a slight change toward either the acid or alkaline side of the usual hydrogen ion concentration of the organism can readily reduce the stability of the tertiary phosphate. It is quite conceivable that abnormalities of diet or certain pathological conditions might bring about such changes. Although this problem will be discussed in detail later (section IX) it is desirable now to mention (*a*) that the deposit of lead retained after the absorption of a lead salt is known to be rather unstable chemically, and (*b*) that this deposit is held within the body where its release would involve the danger of flooding the organism with soluble lead.

The distribution of lead in the human body during lead poisoning. *Analytical studies.* These general conceptions may be applied with interest to the chemical results from human autopsies in cases representing different types and stages of plumbism. Thus far it has been possible to examine in this laboratory for diagnostic purposes the tissues from seven necropsies. In these few investigations we have obtained cases typical of different types of exposure to lead and representing various stages of absorption. The analytical data are collected in table 18 merely to save space. The group should not be studied as a whole for each individual distribution picture and its case history should be considered separately. For complete histories reference must be made to our original report (309). Only the following brief summaries of the cases will be included here.

Case I. A mentally defective child with a perverted appetite gnawed the paint from her bed for several weeks. Gradually severe lead poisoning developed. The symptoms were marked constipation, vomiting, loss of appetite, lead line, marked anemia and stippling of red cells, increasing weakness, tremor, drowsiness, and finally convulsions which developed and were continuous the day before death. The presence of acetone and diacetic acid in the urine indicated a condition of acidosis which was further evidenced by the rather marked odor of acetone in the tissues at autopsy.

Case II. A man aged forty-two had been exposed to lead since a small child when he watched his father spray trees with arsenate of lead. For

TABLE 18

The distribution of lead in human tissues

TISSUE	CASE I		CASE II		CASE III		CASE IV		CASE V		CASE VI		CASE VII	
	Conc.*	Total†	Conc.	Total	Conc.	Total	Conc.	Total	Conc.	Total	Conc.	Total	Conc.	Total
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Liver	5.11	22.99	0.015	0.23	0.12	1.65	0.68	10.88	0.13	1.95	0	0	0	0
Kidney	1.00	0.97	0.09	0.30	0.35	0.94	2.45	7.84	0.39	0.88	0	0	0	0
Spleen	1.07	0.77	Trace	—	1.59	2.23	0	0	0	0	0	0	0	0
Pancreas	10.00	1.09	0.25	0.20	—	—	—	—	—	—	0	0	0	0
Washed gastro-intestinal tract	0.46	1.59	0.01	0.26	—	—	—	—	—	—	0	0	0	0
Heart	0.56	0.30	Trace	—	0.32	0.99	—	—	0	0	0	0	0	0
Lung	0.32	0.61	0.01	0.17	0.29	4.41	0	0	0	0	—	—	—	—
Cerebrum	0.36	2.91	0.02	0.31	0	0	0.22	3.20§	—	—	—	—	0	0
Cerebellum	0.62‡	0.65‡	0.17	0.20	0.49	0.71	0.30	—	—	—	—	—	0	0
Peripheral nerves and cord	—	—	Trace	—	—	—	—	—	—	—	—	—	—	—
Skeletal muscle	—	—	0	0	—	—	—	—	—	—	—	—	—	—
Skeleton	15.30	195.8	4.05	390.0	2.24	280.0	7.16	800.0	9.36	897.0	2.17	243.0	2.47	195.0
Blood	Trace	—	—	—	0.27	—	—	—	—	—	—	—	—	—
Bile	Trace	—	—	—	0	0	—	—	—	—	—	—	—	—
Gastro-intestinal contents	—	—	—	0.17	—	0.90	—	—	—	—	—	—	—	—
Bladder contents	Trace	—	—	—	—	—	—	—	—	—	—	—	—	—

* Conc. = mgm. of lead per 100 gm. of fresh tissue.

† Total = mgm. of lead in whole organ. In many cases this figure is only approximate.

‡ Medulla analyzed with cerebellum.

§ Calculated for entire brain.

twenty-seven years he worked in the forestry department himself, using lead arsenate as a spray, and during each spraying season had severe colic. Double wrist drop had persisted for twelve years in spite of treatment, and during the last three years there was difficulty in walking. After a month's illness death was caused by pneumonia in mid-winter several months after the last exposure to lead. He had a positive Wassermann reaction.

Case III. A man twenty-five years old had never been exposed to lead before working in the mixing room of a rubber company for the seven months preceding death. This room was very dirty and the workmen were exposed to much dust containing lead compounds. Marked constipation, lead line, abdominal pains, and occasional attacks of dizziness appeared during the last five months of life while the man was employed at this trade. Nine days before admission to the hospital his left eye suddenly became blind and the left arm and leg were paralyzed. This condition was only temporary and the patient continued to work until nine days later when he fell to the floor and became very violent within a few minutes. He was at once taken to a psychopathic hospital. Clonic convulsions continued until he died two days later.

Case IV. A man aged sixty who had been a painter for six years was killed while at work by a fall. He had always been well and there had been no symptoms of plumbism.

Case V. A janitor sixty-three years old who had frequently done painting died after an illness of ten weeks, which was diagnosed as coronary thrombosis and auricular flutter. There were no signs or symptoms of lead poisoning but the case was studied on account of the history of exposure to lead.

Case VI. A man of fifty-one had been a stereotyper for twenty-five years. This necessitated working in a small room over vats of molten metal (lead, antimony, and tin) from which fumes rose constantly. Loss of appetite, constipation and loss of weight were noted during the last nine months of life, but these symptoms were probably not due to lead. The blood showed no anemia or stippling. Death was due to an eroding carcinoma of the esophagus which finally produced two sinuses, one leading into the trachea and the other into the left lung.

Case VII. A man aged fifty-three years had been a paint mixer for fifteen years. His health had been good until about nine months before

death. Such symptoms indicative of lead poisoning as abdominal cramps, pallor, lead line and a rapid loss of weight were then noted but were not severe. The blood was markedly anemic, suggestive of a primary type, and no stippling of red cells could be seen. Death was due probably to pernicious anemia.

Discussion. Correlation of these analytical data and the clinical histories demonstrates that events follow the same course in both man and animals after the absorption of lead. The distribution of lead in Case I is quite similar to that in animals when lead has been administered by mouth until shortly before death, and the clinical history furnishes evidence of a similar type of exposure. The high concentration of lead present in the liver is a sign that in man as in animals much of the lead carried in the portal blood is removed and retained by this organ. Of the lead which escapes the liver and enters the general circulation, a high percentage is retained by the skeleton. While absorption continues, however, lead is also present in the blood stream and in all the tissues of the body. In Cases II and III the lead was also well distributed throughout the organism. In Case II there had been no exposure to lead for about six months —a fact corroborated by the absence of any accumulation of lead in lungs or liver. Probably, therefore, the acute infection—pneumonia—and the inability to take adequate food during the period of illness had mobilized from the skeletal store the small amounts of lead which were distributed throughout the organism. In Case III, on the contrary, the relatively high concentration of lead in the lungs as compared with that in the liver indicates that absorption through the respiratory tract was active at the time of death. Further evidence of this is offered by the general distribution of lead in the tissues and by the high concentration in the blood. The truth of these deductions is borne out by the history of recent work at a very dusty trade. Case IV is chiefly interesting because it probably portrays conditions typically found in lead workers without symptoms of plumbism. The chief features of this case are the storage of considerable quantities of lead in the bones, the rather high concentration of lead in the liver, indicative of continued gastro-intestinal absorption, and the presence of lead in the kidney and central ner-

TABLE 19

Miscellaneous analyses of human tissues and fluids

TISSUE	WEIGHT ANALYZED	LEAD FOUND	LEAD IN 100 GRAMS	PATIENT	REMARKS
	grams	*mgm.*	*mgm.*		
Teeth	3.6	0.23	6.37	D. M.	Under treatment for chronic plumbism. Removed for several weeks from exposure to lead
	17.0	3.36	19.75	B. L.	Chronic lead poisoning. Teeth removed after six weeks hospital treatment
	12.86	0.66	5.08	T. B.	During hospital treatment about one month after removal from exposure to lead
Bone graft	2.5	0	0	B. L.	
Bile	175 cc.	0	0	J. L.	Duodenal lavage in case of chronic plumbism
	113 cc.	0	0	S. S.	Duodenal lavage in case of chronic plumbism
	125 cc.	Microchemical trace		M. M.	Duodenal lavage in case of chronic plumbism
Spinal fluid	80.0	0.08	0.10	J. T.	During an attack of acute encephalopathy
	5.0	0	0		Child with lead encephalopathy
Hair	0.50	Trace; micro +		J. S.	After five months hospital treatment for chronic plumbism
	2.57	Micro +		J. T.	One month in hospital after exposure to lead
	4.5	0	0	P. M.	After three months treatment
	5.8	Micro +		P. M.	Six months after admission to hospital
	1.95	Micro +		J. T.	Four months after admission to hospital
	5.1	0.17	3.33	D. M.	Three months after admission to hospital
Saliva	50.0	0	0	M. R.	Case of chronic plumbism on admission to hospital

vous system—a sign that the process of transportation of lead was active despite the absence of symptoms.

In Cases VI and VII, on the other hand, the lead was entirely localized in the skeleton. Here absorption had not taken place for some time and apparently the stored lead had not been mobilized. The distribution of lead in such cases is completely analogous to that in animals which have been killed long after exposure; significant quantities of lead are to be found only in the skeleton.

A few determinations of isolated tissues and fluids from cases of plumbism have been made and seem of sufficient interest to include (see table 19). A concentration of lead similar to that found in the rest of the bony tissue is present in teeth and suggests a possible way of proving previous lead absorption. Though lead was frequently detected in hair, there was no great concentration to indicate a special localization of lead in this tissue as suggested by Meillère (292).

Medico-legal significance of analytical findings. Since the additional evidence furnished by the chemical analysis of tissues is frequently required to settle medico-legal questions arising in cases of plumbism, it is of interest to consider what conclusions may justifiably be drawn from the different types of distribution pictures. When lead appears exclusively in the bone it must have been absorbed at some previous time, but mere storage in the skeleton is no evidence that lead was involved as a cause of death. Similarly, if lead is present only in the gastro-intestinal tract or its contents, it may merely have been swallowed and not absorbed. On the other hand, the presence of lead in the urine or in a majority of the tissues is a sign of active transportation of lead by the blood stream. This is evidence of either recent absorption or mobilization of stored lead from the bones. In either case danger of the exposure of the tissues to soluble lead is obvious, and lead may then rightfully be considered at least a complicating factor in the pathological picture.

Summary and conclusions. Studies of the distribution of lead in the tissues of man and animals during plumbism show the same general course of events. Early in lead absorption differences in distribution are found dependent upon the route by which lead has entered the organism. Following absorption from the gastro-intestinal tract, the liver contains a relatively high concentration of

lead due to a retention of this toxic substance which it removes from the portal blood. From the lung or subcutaneous tissue, on the contrary, lead enters the general circulation and, hence, only a small portion of it is carried directly to the liver. Once in the general circulation the lead is distributed throughout the organism. Since the skeleton is the only tissue to retain permanently any significant amount of lead an ever increasing percentage of the total absorbed lead comes to be stored in the bones. After absorption has ceased, this selective localization of lead becomes practically complete. While stored in such a deposit the lead is apparently harmless, since the symptoms of plumbism are noted only when lead is generally distributed throughout the organism, as a result either of recent absorption from an external source, or of mobilization of a skeletal store.

VII. EXCRETION OF LEAD

After absorption into the general circulation, lead is either stored in the skeleton or eliminated from the organism by excretion. To complete our knowledge of the course which lead follows from the time it is absorbed until it finally leaves the body, it remains to consider the various excretory routes and to study the factors controlling elimination and storage. Of the three paths of excretion—the alimentary canal, the kidney, and the skin—that first recognized was the gastro-intestinal tract. Although the very early treatment of lead poisoning by drastic purging seems to imply a knowledge of the excretion of lead in the feces, accurate information could not be obtained until methods of chemical analysis were devised. As early as 1839 Devergie (96), in collaboration with Tanquerel des Planches (453), definitely detected lead in the tissues and contents of the gastro-intestinal tract and also in the kidneys and bladder of individuals suffering from lead poisoning. Since that time analysis of the excreta has frequently accompanied investigations of other phases of chronic plumbism. For the sake of clarity each of the paths of excretion will be discussed separately here.

Excretion by the gastro-intestinal tract. In spite of the early recognition of the gastro-intestinal tract as an important path for the elimination of lead from the organism, there are still many features

of the mechanism which are but little understood. Ever since Tanquerel's time there has been no doubt that lead is eliminated in the feces; and detailed information about the comparative activity of various parts of the gastro-intestinal tract and the exact process of excretion has gradually accumulated, until there is a general belief that the entire alimentary tract excretes as well as absorbs lead (293) (39). Blum (39) considered the lead line a sign of excretion in the saliva; Pouchet's (365) experimental observation that lead is present in the saliva of patients with chronic plumbism, even after exposure has ceased, has not been confirmed in this laboratory in a patient undergoing treatment for chronic lead poisoning. Renon (382) found lead in the salivary glands of experimentally poisoned animals. That lead should appear in the saliva or salivary glands while it is being transported in the blood is not surprising, although excretion by this path would be rendered quite ineffective by re-absorption of the lead in other parts of the tract.

No conclusive data regarding the rate of the excretion of lead in different portions of the gastro-intestinal tract are available because of the difficulty of obtaining such information in an intoxication as slow as chronic plumbism. As a natural result of the early observation that following lead ingestion relatively large quantities of lead are picked up and held by the liver, excretion in the bile has received much more attention than elimination by any other route.

Tanquerel's (453) attempts to demonstrate the presence of lead in bile were inconclusive. Although Hermann (209) (1874) really only assumed that lead is excreted in the bile after detecting it in both the liver and the small intestine, he is generally credited with having furnished final proof. The first real experimental work on the subject was contributed by Annuschat (9) (1877). He fed lead to rabbits with biliary fistulae, and found considerable quantities of lead in the bile in acute stages of poisoning; in the more chronic stages little or none, although the liver contained comparatively large amounts. Lehmann (257), on the other hand, in the controversy with Annuschat found that during the chronic poisoning following subcutaneous injection, the concentration of lead in the bile was higher than that in the liver; and he believed that the liver collects the lead to be poured out in the bile. Lavrand (248) (1886) likewise believed that bile is the main path of excretion for lead. Harnack (200) in 1897 contributed

the information that the proportion of lead excreted in the bile depends upon the portal by which it enters the body—that it is greatest following gastro-intestinal absorption. Several more recent investigators have repeated these observations without adding any really new information (293) (252). Siccardi and Roncato (428) in 1912 attempted to explain the mechanism involved in excretion of lead by the liver by a suggestion which

TABLE 20

Excretion of lead in bile and gastro-intestinal tract

NUMBER OF RABBIT	AMOUNT OF LEAD GIVEN	METHOD OF ADMINISTRATION	INTERVAL BEFORE COLLECTION	LENGTH OF COLLECTION PERIOD	INTERVAL BETWEEN INJECTION AND DEATH	TOTAL PB IN BILE	AVERAGE HOURLY EXCRETION OF LEAD IN BILE	LEAD FOUND IN GASTRO-INTESTINAL TRACT			LEAD IN THE LIVER
								Stomach	Small intestine	Colon	
	mgm.		*hours*	*hours*	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
701	125.0	Jugular vein	0	9	9	1.87	0.21	—	—	—	—
702	110.5	Jugular vein	0	14.5	14.5	1.16	0.08	1.01	1.81	0	56.45
703	130.0	Jugular vein	0	4	11.	0.57	0.14	0.58	0.0	1.21	39.31
704	111.6	Jugular vein	0	3	4.75	0.55	0.18	0.62	0.59	—	—
705	116.5	Jugular vein	0	8.5	8.5	1.78	0.21	0.38	1.22	0.61	35.20
706	93.2	Jugular vein	0	2.5	7	2.52	1.00	2.30	0.92	1.08	36.20
708	69.0	Jugular vein	0	4	4	1.28	0.32	0.58	1.72	0.45	37.71
722	546.0	By mouth	3	3		0.43	0.14				11.19
721	546.0	By mouth	3.5	3		0.72	0.24				
723	977.0	By mouth	3	3		0.82	0.27				
725	977.0	By mouth	2	3.5		0.09	0.03				
726	546.0	By mouth	3	3		0.11	0.04				
720	1,000.0	By mouth	18	2		0.40	0.20				

Average hourly excretion in bile for all animals, 0.23 mgm.

involved impossible chemical reactions. According to them, lead is brought to the liver by leucocytes, detoxicated by the Kupfer cells which reduce it to the metallic state, and in this form is excreted in the bile.

In a series of unpublished experiments, Brady and others in this laboratory have studied the biliary excretion in rabbits following administration of a comparatively large single dose of lead either intravenously or by mouth. The bile duct was cannulated, the bile collected for several hours, and its lead content determined. From these data the average hourly excretion of lead could be calculated (see table 20). Since the bile duct was cannulated before injection, detection of lead in the gastro-intestinal tract indicated

that there was simultaneous independent excretion in the bile and by the gastro-intestinal tract. In comparing the amounts of lead excreted by these two paths (see table 20) it must be borne in mind that the lead in the stomach and in the intestines was excreted during the entire interval between the injection of lead and death, while the specimen of bile examined was generally collected during a much shorter period. The average rate of excretion of lead in the bile of these animals, 0.23 mgm. per hour, is undoubtedly higher than in more chronic cases since the larger single dose acutely flooded the organism just before the specimens were collected. But the data serve (*a*) to confirm the earlier reports of an excretion of lead in the bile, and (*b*) to demonstrate an independent excretion of lead in the stomach and intestines.

Our information regarding the excretion of lead by the gastro-intestinal tract as a whole may be summarized as follows: (*a*) The entire alimentary canal probably excretes lead; (*b*) the bile is an important factor in this excretion, especially following gastro-intestinal absorption; (*c*) the gastro-intestinal tract is generally considered the chief route by which lead is excreted.

Excretion of lead by the kidney. The unreliability of the methods of chemical analysis employed, and the fact that different types and stages of plumbism were studied, undoubtedly account for the variety of opinion among earlier investigators regarding the excretion of lead by the kidney.

For instance, Tanquerel (453) failed to find lead in the urine, while Melsens (297) somewhat later demonstrated its presence. Lavrand (248) in 1886 reported that lead was to be found in the urine of patients with plumbism only after medication. Murgia (323), on the other hand, in 1912 states that the appearance of lead in the urine is the earliest and most constant sign of lead poisoning. Among the more modern authorities, Legge and Goadby (252), Oliver (337), and Meillère (293) agree that the urine in cases of chronic plumbism frequently contains lead, but that the kidney excretes far less than does the gastro-intestinal tract.

Two groups of experiments have been carried out in this laboratory to furnish further correlation between the urinary and fecal excretion of lead. First, a few single determinations of the daily excretion in cats by these two routes were made in order to determine their relative importance following the entrance of lead into the organism by various portals. The figures,

collected in table 21, agree with the reports of earlier investigators. As would be expected, large quantities of lead were excreted in the feces after administration by mouth.

As Oliver (337) (page 161), Legge and Goadby (252) (page 19), and others have pointed out, however, probably only a small percentage of this apparently excreted lead had been absorbed, and the

TABLE 21

Excretion of lead in the urine and feces of cats

NUMBER OF CAT	SPECIMEN NUMBER	DAILY EXCRETION IN URINE	AVERAGE DAILY FECAL LEAD EXCRETION FOR PERIOD INCLUDING DAY OF URINE ANALYSIS
a. During lead feeding			
		mgm.	*mgm.*
149	1	0	5.91
	2	0	23.70
	3	0.34	36.07
	4	0.22	17.70
	5	0.76	27.90
179	1	0	63.77
	2	0	48.84
148	1	0	170.00
b. After subcutaneous administration of lead			
292	1	0	3.13
	2	0.23	0.15
296	1	0.48	0.31
	2	0.56	1.06
286	1	0.36	0.30
	2	0.42	0.16

remainder had merely passed unchanged through the gastro-intestinal tract. Moreover, much of the lead absorbed was probably removed from the portal blood by the liver and excreted without ever reaching the general circulation. Thus, lead in the feces following the ingestion of lead compounds is no real index of the amount of lead actually entering the organism, but the small amounts of lead occasionally found in the urine indicate that lead is being transported in the blood

stream. When all the lead absorbed enters the general circulation directly—a condition which can be produced experimentally by subcutaneous injection of lead—excretion is much more gradual. Several factors explain this difference: (*a*) The fact that unabsorbed lead cannot be excreted from a subcutaneous deposit, (*b*) the slower entrance of lead into the blood stream, and (*c*) the tendency of the

TABLE 22

Comparative rates of excretion of lead in urine and feces of man

PATIENT	NUMBER OF CONSECUTIVE THREE-DAY PERIODS INCLUDED IN AVERAGE	AVERAGE EXCRETION PER PERIOD		RATIO Urine : Feces
		Urine	Feces	
		mgm.	*mgm.*	
J. S.	5	0.23	0.39	1:1.7
J. S.	2	0.43	1.15	1:2.7
R. D. W.	3	0.28	1.10	1:4.0
J. L.	1	0.78	0.96	1:1.2
J. L.	1	0.26	0.42	1:1.6
D. M.	4	0.46	0.97	1:2.1
D. M.	4	0.29	0.85	1:3.0
P. M.	2	0.15	0.06	1:0.4
P. M.	2	0.45	0.17	1:0.4
P. M.	4	0.30	0.78	1:2.6
P. M.	4	0.25	1.04	1:4.0
P. M.	3	0.26	0.98	1:3.8
J. T.	2	0.72	1.56	1:2.1
J. T.	2	0.58	1.86	1:3.2
J. T.	4	0.38	0.29	1:0.8
J. T.	4	0.23	1.11	1:4.8
M. M.	1	0.12	0.51	1:4.0
Average				1:2.5

skeleton to remove and retain circulating lead. Although there is not only an absolute but also a relative decrease in the quantity of lead excreted in the feces after subcutaneous injection, the rate of excretion by this route is generally greater than that by the kidney.

To furnish comparative data on elimination of lead in urine and feces, observations made on patients with chronic plumbism proved more satisfactory than those on animals. The subjects were under observation in a hospital and analyses were not made until they had been removed from exposure to lead for some time. For chemical analysis,

the urine and feces were collected separately in three-day periods marked by the administration of carmine. The results presented in table 22 show that there is no definite quantitative ratio between urinary and fecal excretion. Much more lead is eliminated by the gastro-intestinal tract than by the kidney, although in six of the periods included in the averages shown in table 22, small amounts of lead appeared in the urine when none could be detected in the feces. This is unusual, however, and in these observations the average ratio between the amounts excreted in urine and feces is 1:2.5. More striking and significant, however, is the regularity of the urinary excretion, which never becomes great yet was negative in only 5 of the 48 periods studied. Thus the extreme ranges of urinary excretion for three-day periods in all the cases studied were from 0 to 0.96 mgm. of Pb, while the corresponding elimination in the feces varied from 0 to 2.05 mgm. The averages as presented in table 22 naturally vary much less—from 0.12 to 0.78 mgm. of Pb in urine, and from 0.06 to 1.86 mgm. in feces. Obviously the excretion of large amounts of lead must be accomplished by the gastro-intestinal tract, and therefore the greater the total output of lead the greater becomes the disproportion between the quantities of lead eliminated in urine and feces.

This simple observation that the amount of lead which the kidney can excrete is distinctly small, while that which the gastro-intestinal tract can eliminate is unlimited, probably depends upon very fundamental factors of excretion. An analogy certainly seems to exist between the excretion of lead and calcium. It has frequently been observed that a high phosphate diet tends to increase the proportion of calcium excreted in the feces, while the ingestion of hydrochloric acid, on the contrary, increases the urinary output. Although the reasons for this have not been clearly worked out, a possible explanation may be found in the relative solubility of the compounds formed. Thus calcium chloride, a soluble salt, appears in the urine, while calcium phosphate, which is very insoluble, is excreted almost entirely by the intestine. As has been shown in section IV, lead exists and is transported in the organism chiefly in the form of highly dispersed colloidal lead phosphate, which is very insoluble. The amounts of this which may be dissolved are small and vary with the hydrogen ion

concentration in the organism. The fact that the acidity is always very low may possibly explain the limited excretion of lead in urine.

Excretion of lead by the skin. The observation that the skin of patients suffering from plumbism can be darkened by exposure to hydrogen sulphide gas has been considered by some investigators as evidence of cutaneous absorption of lead, and by others as proof of cutaneous excretion. Du Moulin's early work was verified by Lavrand (248) who carried out extensive studies showing that much of the coloration produced by exposure to hydrogen sulphide is due to the formation of *iron* and not *lead* sulphide. Furthermore, Lavrand found that as time elapsed after exposure, the degree of coloration gradually became less—an indication that the lead present soon after exposure is merely contamination and has not been excreted. The same criticism may probably also be applied to Oliver's (337, page 115) observation that lead can be detected in perspiration; and likewise to the findings which led Meillère to believe that lead is excreted not *by* but *to* the skin, hair, and other keratinous tissue where it is stored in some harmless chemical combination (292). The figures already presented in chapter VI which show the absence of any accumulation of lead in the hair or skin are evidence against this idea of an "internal excretion" of lead to the keratinous tissue.

Summary. The more important points regarding the excretion of lead are that:

Lead is excreted by the entire gastro-intestinal tract, in the bile, by the kidney, and possibly also to a slight extent by the skin.

By far the greatest proportion of lead is excreted in the feces, though small amounts are usually eliminated simultaneously by the kidney.

The small amounts of lead in urine are of much greater clinical and diagnostic significance, for if lead is being ingested it is impossible to determine whether the lead in the feces has been absorbed or has merely passed unchanged through the alimentary canal.

VIII. FACTORS INFLUENCING THE RATE OF LEAD EXCRETION IN ANIMALS

After absorption has ceased, the rate at which lead can be excreted from the organism depends chiefly upon the stability of the skeletal store. Since both lead and calcium are held in the body at a

common site and the chemical behavior of some of their salts *in vitro* is very similar, it seems quite probable that the same physiological conditions might favor the liberation or retention of both. A review of information regarding the chief factors which influence calcium metabolism furnishes many suggestions about a possible control of lead storage.

Calcium excretion. Many different physiological and pathological factors influence the calcium economy of the organism. Most obvious of these in determining whether calcium shall be drawn from or added to the skeletal store is diet. Not only the relation of the actual calcium intake (420) (346) (112) (160) (169) (354) to the requirements of the body, but also the vitamine content (202) (426) (423) (424) (211) (170) (220) and the acid or basic nature of the food (41) (33) (161) (134) play an important part. Perhaps even more potent and fundamental changes are produced within the organism by such conditions as (*a*) acidosis, whether due to excessive fatigue, incomplete oxidation of inorganic acids, or acute infection (436) (397) (186) (329) (498), (*b*) pregnancy and the fetal demands upon the calcium reserve (436) (42) (97) (98), (*c*) lactation, which involves a prolonged loss of calcium (146) (150) (291) (436) (97), and (*d*) variations of endocrine activity (27) (180) (44). Medication also has a profound influence upon calcium metabolism, and since the changes which it produces may be experimentally controlled, it provides a practical means of studying the stability of the lead stored in the organism. The types of such medication which have been most studied fall roughly into groups: (*a*) calcium salts, (*b*) antagonistic cations such as magnesium, potassium, and sodium, and (*c*) acids and alkalies. In addition to these, sunlight has been found to produce marked effects.

The administration of calcium salts does not invariably change the calcium balance toward the positive side as might be expected. Berg (33) found that ingested calcium phosphate adds nothing to the calcium store since it is merely excreted unchanged in the feces; and this is in accord with the observations of Étienne (123) and Bonnamour, Sarvonat *et al.* (44), who showed that prolonged administration of calcium chloride causes marked decalcification following a slight transient retention. This drain upon skeletal calcium was shown by Étienne to be so severe that deformities resembling osteomalacia developed in his experimental animals. Berg believed that any inorganic anion aids such depletion of the calcium store and advised administration of calcium lactate or bicarbonate to induce a retention. Steenbock, Hart *et al.* (437), however, found that the addition

of calcium lactate, carbonate, chloride, silicate or phosphate to diets adequate in all respects except for their calcium content, maintained normal growth in rats. The decalcifying effect of magnesium, sodium, and potassium salts depends upon the fact that the organism tends to maintain a normal ratio between the concentrations of these various salts in the body fluids. Thus, if one is present in excess, the ratio is restored by calling upon the reserves of the others. Among the salts which have been used to produce decalcification in this way are sodium chloride (333) (207), potassium iodide (134), magnesium chloride and citrate (41), and magnesium oxalate (117). As Berg suggested, part of the efficiency of some of these may be due to the action of inorganic anions, but the antagonistic cations also seem to play a part.

The chemical reasons for the administration of acid to liberate stored calcium are obvious. The solvent action of many acids has been tested and the most effective have proved to be those which are non-toxic and cannot be readily burned in the organism but must be neutralized by the bases present. Of the mineral acids, hydrochloric (438) (159) (163) (174) and phosphoric (ingested either as such or as the acid phosphate) produce marked decalcification (33). Organic acids are much less useful. Sarvonat and Roubier (395) experimented upon animals with oxalic acid and others have frequently used the less toxic and much less effective lactic and citric acids (134). That administration of alkalies would produce an effect quite contrary to that of acids, i.e., would favor retention of calcium, is an apparently reasonable assumption. Introduction of an alkali in the organism, of course, helps neutralize any acid present and so decreases the demand on the basic reserves. But experiments demonstrate that when an excess of alkali in the form of sodium bicarbonate is introduced into a normal individual, there is a tendency toward calcium loss rather than retention (159) (396). The effect of sunlight on calcium metabolism is not understood but it is known that exposure to the sun's rays favors storage of calcium in the bones of children. Sunlight is therefore extensively used as a therapeutic agent in rickets (212) (425).

Lead excretion. This brief survey suggests several logical methods of investigating the factors which control the store of lead within the skeleton. The most practical of these involve variations of diet and medication with acids and salts which liberate reserve stores of calcium. They should theoretically also prove effective in mobilizing lead which has been deposited in the bones. Because of the warning frequently repeated in the literature that administration

of potassium iodide may suddenly liberate stored lead and cause severe intoxication, it has seemed best to carry out experiments with animals before attempting to treat patients with these more powerful decalcifying agents.

In these experiments, cats which had been severely poisoned either by prolonged lead feeding or by the insufflation of lead carbonate, were allowed

TABLE 23

Effect of starvation and hydrochloric acid on lead excretion

NUMBER OF CAT	TREATMENT	WEEKLY DOSE OF MOLAR SOLUTION	CALCIUM INTAKE	WEEKLY EXCRETION OF LEAD	BLOOD CO_2	BRIEF HISTORY
		cc.		*mgm.*	*volume per cent*	
15	Control	0	High	3.64		Received 3.32 grams of lead in 32 doses by mouth from March 9, to May 22, 1922. This observation started November 1, 1922
	Control	0	High	4.69		
	HCl and starvation	43	0	2.24	25.2	
	Control	0	High	2.45		
	Control	0	High	0		
11	Control	0	High	0		Received 15.61 grams of lead in 54 doses by mouth from January 20, to June 8, 1922. This observation started October 1, 1922
	Control	0	High	0		
	HCl and starvation	49	0	8.19	20.7	
	Control	0	High	0*		
	Control	0	High	6.44		
8	Control	0	High	1.48		Received 11.22 grams of lead in 68 doses by mouth from January 20, to June 8, 1922. This observation started September 1, 1922
	Control	0	High	4.27		
	HCl and starvation	52.5	0	6.02	23.6	
	Control	0	High	5.29		
	Control	0	High	0		
	HCl and starvation	42	0	3.99	22.3	
	Control	0	High	0		
	Control	0	High	0.21		

* Very constipated.

to recover completely after the administration of lead had ceased. They were then confined in metabolism cages so that their excreta could be analyzed during control and medication periods. As no attempt was made to separate urine from feces, the figures presented here represent the combined excretion by the two routes. In one series of experiments the animals were

TABLE 24

Effect of ammonium chloride on lead excretion

CAT		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6	PERIOD 7	REMARKS
217	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung January 26, 1923, February 2, 1923, February 12, 1923. This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149.5	112.1	130.8	0	0	
	Pb excreted weekly mgm	1.22	0	0	1.27	0	0.17	1.83	
337	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by mouth October 5, 1922, to March 1, 1923. This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149.5	112.1	130.8	0	0	
	Pb excreted weekly mgm	0	1.07	0.28	1.16	2.88	0	0.25	
305	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung June 29, 1923. This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	130.8	112.1	140.0	0	0	
	Pb excreted weekly, mgm	0	0.24	0.13	2.30	0.74	0.53	2.10	
308	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung June 29, 1923. This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149.5	112.1	140.0	0	0	
	Pb excreted weekly, mgm	0	0.29	0	1.11	0.13	0.29	0	
303	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung June 29, 1923. This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149.5	112.1	149.5	0	0	
	Pb excreted weekly, mgm	0	0	0	1.04	2.77	1.14	0.60	

at first starved during the administration of acid in order to increase the drain upon the calcium reserve by the production of acid within the organism. Later, when longer observation periods were desirable, they received a diet consisting of meat or liver which contain a very little calcium. The nutrition was adequate except for calcium.

The data obtained after administration of HCl demonstrate that the acid caused a prompt increase in excretion (see table 23). The

TABLE 25

The effect of ammonium chloride on lead excretion

CONTROL ANIMALS			TREATED ANIMALS		
Number of period	Weekly excretion of lead		Number of period	Weekly excretion of lead	
	Cat 284	Cat 238		Cat 478	Cat 495
	mgm.	*mgm.*		*mgm.*	*mgm.*
1	0	11.23	1	5.57	4.90
2	2.87	3.31	2	1.96	4.69
3	2.17	1.91	3	4.47	1.84
4	0	0	4	3.52	3.44
5	0	0	5	2.26	0.12
6	0.17	0	6	3.17	3.50
7	0.17	0	7	4.28	2.40
8	0.73	6.63*	8	5.08	5.07
9	0	0.25	9	4.92	7.70
Average...	0.68	2.59	Average....	3.91	3.74
Average for two controls.....1.63			Average for two treated animals...3.82		

* Animal sick with snuffles.

low capacity of blood plasma for CO_2 is evidence of a considerable degree of acidosis. Although acid may be thus ingested in effective doses (in cats by stomach tube) it frequently causes nausea and vomiting, and large doses cannot be taken without gastric disturbance.

J. B. S. Haldane (183) has recently reported that rather large doses of ammonium chloride cause marked acidosis in man without digestive disturbances. Since this salt liberates one molecule of hydrochloric acid when 2 molecules of ammonia combine with carbon dioxide to form urea in the body, a means is provided of administering hydrochloric acid indirectly. Although in very large doses

TABLE 26

Effect of phosphoric acid on lead excretion

	TREATMENT	WEEKLY DOSE OF MOLAR SOLUTION	CALCIUM INTAKE	WEEKLY EXCRETION OF LEAD	BRIEF HISTORY
			Cat 217		
		cc.		*mgm.*	
	Control	0	High	0	Received ±2 cc. of lead carbonate suspension by lung on January 26, February 2, and February 12, 1923
	Control	0	Low	0.12	
A	H_3PO_4	32.0	Low	0.27	
	H_3PO_4	38.2	Low	0.40	Observation A started May 17, 1923
	Control	0	High	0.21	
	Control	0	High	0.72	Observation B started April 30, 1924
	Control	0	High	3.22	
B	H_3PO_4	82.7	Low	4.78	
	H_3PO_4	63.6	Low	3.67	
	Control	0	High	1.47	
	Control	0	High	1.69	
			Cat 337		
	Control	0	High	0	Received 7.7 grams of lead as lead acetate solution by mouth from October 5, 1922, to March 1, 1923
	Control	0	Low	0.16	
A	H_3PO_4	25.4	Low	0.50	
	H_3PO_4	38.3	Low	1.22	Observation A started May 17, 1923
	Control	0	High	1.44	
	Control	0	High	1.56	Observation B started March 23, 1924
	Control	0	High	0.69	
B	H_3PO_4	82.7	Low	4.12	
	H_3PO_4	63.6	Low	3.01	
	Control	0	High	0.24	
			Cat 305		
	Control	0	Low	0.25	Received ±2 cc. of lead carbonate suspension by lung June 29, 1923
	Control	0	High	0	
A	H_3PO_4	93.8	Low	0	
	H_3PO_4	66.1	Low	1.26	
	Control	0	High	0	Observations A and B are continuous and started September 2, 1923
	Control	0	High	0	
B	H_3PO_4	76.5	Low	1.36	
	H_3PO_4	114.7	Low	1.70	
	Control	0	Low	0	
	Control	0	High	0.50	

TABLE 27

Effect of di-ammonium phosphate on lead excretion

CAT		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6	REMARKS
238	Calcium diet	High	Low	Low	Low	High	Low	Leaded by lung November 5, 1923. This observation started January 4, 1924
	M/1 solution weekly, cc	0	45.4	49.2	68.2	0	0	
	Pb excreted weekly, mgm	0.25	4.92	3.71	1.57	1.57	0.39	
284	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung November 5, 1923. This observation started December 28, 1923
	M/1 solution weekly, cc	0	0	45.4	49.2	68.2	0	
	Pb excreted weekly, mgm	0.73	0	0.29	0.19	0	0.40	
600	Calcium diet	High	Low	Low	Low	High	High	Leaded by lung November 5, 1923. This observation started December 28, 1923
	M/1 solution weekly, cc	0	0	49.2	68.2	0	0	
	Pb excreted weekly, mgm	0.48	1.35	0.27	1.99	0.38	0.87	

it is an emetic and the amount which can be administered to animals is therefore somewhat limited, doses which can be tolerated increase the excretion of lead when the diet contains little calcium (table 24).

Other experiments were also performed to demonstrate the effect of ammonium chloride (112 cc. M/1 solution per week). Four animals were given lead intratracheally, and while absorption was still going on, two of them were treated with daily doses of ammonium chloride. The animals receiving ammonium chloride excreted approximately twice as much lead as did the controls and provide an excellent illustration of the action of this drug in preventing complete storage of lead in the bones (see table 25).

Phosphoric acid is less highly dissociated than hydrochloric and consequently can be tolerated in higher concentration. It causes vomiting less frequently and increases the rate of elimination of lead in cats very promptly (table 26). A few experiments performed to test the acid-forming effect of di-ammonium phosphate demonstrated that doses which can be tolerated produce rather variable changes. Although the figures (see table 27) show that the excretion of lead increased slightly, this salt was quite ineffective in two of the three cases studied. Later work with man has confirmed this observation.

Since tartaric acid is a well-known solvent for lead phosphate *in vitro*, it also was administered to our animals. It proved toxic, however, especially to the kidney, and did not favor elimination of lead to any considerable degree.

Observations on the effect of sodium bicarbonate have proved most instructive in pointing out the essential mechanism of the action of acids and alkalies upon stored lead. When administered to a starving animal with acidosis sufficient to mobilize the stored lead, sodium bicarbonate favors storage of lead (310) probably by restoring the normal hydrogen ion concentration of the organism. But when given in excessive doses to an animal normal except for a deposit of lead in the skeleton, sodium bicarbonate tends to increase greatly the elimination of lead (see table 28). The chemical explanation of these apparently opposite effects is suggested by the work of Fairhall and Shaw (129) (see section IV). Their work suggests that while lead phosphate is least soluble within the usual range of hydrogen ion concentrations in the organism, a relatively slight increase of either acidity or alkalinity promptly increases its solu-

TABLE 28

Effect of sodium bicarbonate on the excretion of lead

CAT		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6	REMARKS
238	Calcium diet	High	Low	Low	Low	Low	High	Leaded by lung November 5, 1923. This observation started February 1, 1924
	M/1 solution weekly, cc.	0	0	119	119	0	0	
	Pb excreted weekly, mgm.	1.57	0.39	2.63	1.41	1.93	0.75	
284	Calcium diet	High	Low	Low	Low	Low	High	Leaded by lung November 5, 1923. This observation started February 1, 1924
	M/1 solution weekly, cc.	0	0	119	119	0	0	
	Pb excreted weekly, mgm.	0.40	3.03	1.86	2.13	2.60	0	
600	Calcium diet	High	Low	Low	Low	Low	High	Leaded by lung November 5, 1923. This observation started February 1, 1924
	M/1 solution weekly, cc.	0	0	119	119	0	0	
	Pb excreted weekly, mgm.	0.38	0.87	4.69	2.18	0.21	0.33	
337	Calcium diet	High	High	Low	Low	Low	High	Leaded by mouth. Total 7.7 grams. Last dose March 11, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc.	0	0	142.8	95.2	0	0	
	Pb excreted weekly, mgm.	0	0.25	0	1.32	1.24	2.57	
217	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung January 26, 1923, February 2, 1923, February 12, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc.	0	0	142.8	95.2	0	0	
	Pb excreted weekly, mgm.	0.17	1.83	1.38	5.77	5.07	4.13	
303	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung June 29, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc.	0	0	142.8	95.2	0	0	
	Pb excreted weekly, mgm.	1.14	0.60	2.36	4.11	3.17	1.83	
305	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung June 29, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc.	0	0	142.8	95.2	0	0	
	Pb excreted weekly, mgm.	0.53	2.60	0	1.78	2.35	0.22	
308	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung June 29, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc.	0	0	142.8	95.2	0	0	
	Pb excreted weekly, mgm.	0.29	0	6.25	6.18	5.78	0.73	

bility. It seems possible therefore that acids and alkalies liberate lead by shifting the hydrogen ion concentration, perhaps only locally, to a point sufficiently acid or alkaline to increase the solubility and permit liberation of some of the stored lead phosphate.

Too few animals have been studied and too many complicating factors are involved to warrant any conclusions as to the relative efficiency of the different forms of medication in ridding the organism of lead, but the data do show that decalcifying agents tend to set free lead without—in so far as can be judged from animal experiments—producing any alarming symptoms of lead intoxication. The slight loss of appetite and general malaise which appear during this forced treatment promptly disappeared as soon as medication ceased. Among the animals to which acid was administered while lead was being actively absorbed from the lungs, there were a few exceptions to this. The acid seemed to hasten absorption as well as to liberate stored lead. If medication was continued under these conditions the poisoning sometimes became fatal. This corresponds to the results obtained by Melsens (298) and others when poisoned animals were treated with potassium iodide. The appearance of a lead line, marked loss of appetite, increasing weakness and constipation, however, usually gave ample warning that the organism was being overwhelmed by lead, and symptoms quickly disappeared if medication was stopped and the calcium intake increased. From the point of view of safety, therefore, the use of these decalcifying agents to increase the elimination of lead in man seemed justified.

To determine how completely retained lead can be removed from the organism by reasonably prolonged medication, the tissues of these poisoned animals were analyzed for lead after treatment. The data from thirteen cats are presented in table 29. Comparatively large amounts had been eliminated by these cats. For example, in the periods determined for cat 217, 91 mgm. of lead had been excreted over a period of four hundred and twenty days of observation, and for cat 337, after lead by mouth, 51 mgm. in three hundred and fifty days. However, considerable quantities were still stored in most cases after prolonged periods of observation. The detection of unabsorbed lead in the lungs of the cats which had received lead by insufflation of course indicates that continued absorption may have

TABLE 29

Analysis of tissues of cats after prolonged medication

NUMBER OF CAT	METHOD OF LEAD ADMINISTRATION	APPROXIMATE AMOUNT OF LEAD GIVEN	INTERVAL BETWEEN DOSE AND DEATH	TOTAL LEAD IN BODY*	PER CENT OF TOTAL IN SKELETON	UNABSORBED LEAD STILL IN LUNG	NUMBER OF MEDICATION PERIODS	MEDICATION RECEIVED
		mgm.	*days*	*mgm.*		*mgm.*		
8	By mouth	11,200	244	23.33	97.3		3	HCl (2) Lactic and phosphoric acid (1)
11	By mouth	15,610	172	108.86	98.5		2	HCl (2)
15	By mouth	3,320	307	49.82	99.3		4	HCl (1); lactic acid (2) H_3PO_4 (1)
337	By mouth	7,700	506	35.28	96.8		19	Tartaric acid (2); H_3PO_4 (6) NH_4Cl (7); thyroid extract (2); $NaHCO_3$ (2)
217	By lung	600–700	532	71.14	100.0	3.78	21	KCl (2); KI (2); lactic (2) H_3PO_4 (8); NH_4Cl (5); $NaHCO_3$ (2)
305	By lung	250	390	9.19	100.0	0.56	11	H_3PO_4 (6); NH_4Cl (3) $NaHCO_3$ (2)
306	By lung	250	173	38.37	97.7	7.37	8	H_3PO_4 (6); NH_4Cl (2)
308	By lung	250	387	21.40	100.0	3.20	13	HCl (2); lactic (2); HCl (2); NH_4Cl (3) $NaHCO_3$ (2); H_3PO_4 (2)

238	By lung	250	259	35.41	100.0	5.35	7	$(NH_4)_2HPO_4$ (3); $NaHCO_3$ (2) H_3PO_4 (2)
284	By lung	250	265	10.43	100.0	4.88	7	$(NH_4)_2HPO_4$ (3); $NaHCO_3$ (2) H_3PO_4 (2)
600	By lung	250	173	25.52	96.5	11.50	6	$(NH_4)_2HPO_4$ (2); $NaHCO_3$ (2); H_3PO_4 (2)
478	By lung	300	197	30.74	100.0	9.00	8	NH_4Cl (8)
495	By lung	300	197	12.84	100.0	0	8	NH_4Cl (8)

* Exclusive of lung in cases receiving lead by insufflation.

been partly responsible for the lead remaining in the tissues despite medication. The quantity of retained lead in these animals, however, is not greater than in those receiving lead by mouth. In the latter, absorption must have ceased within a few days after the last dose was given. While these results on the whole discourage the hope of completely freeing the organism from lead, experiments on man in which subjective symptoms can be accurately determined alone can decide the relative desirability of (*a*) favoring complete storage of lead in the bones by high calcium intake and a careful maintenance of a normal acid-base equilibrium, or (*b*) eliminating as much lead as possible by medication before establishing these measures to insure storage of the remaining lead.

Summary. It has been shown by chemical studies with animals that an analogy exists in the metabolism of calcium and lead. Various decalcifying agents have been shown also to increase the lead output. Conversely, conditions favoring calcium retention also tend toward a complete storage of lead in the bones.

Although the lead elimination had been considerably increased, the analysis of the tissues of animals after prolonged medication showed significant amounts of lead still retained by the bones. While it is probably impossible to "de-lead" a poisoned individual completely, further experiments on man alone can demonstrate the relative desirability of a preliminary medication to increase the excretion of lead or of working from the first for a complete storage of lead in the skeleton.

IX. FACTORS INFLUENCING THE RATE OF LEAD EXCRETION IN MAN

The experiments with animals have indicated that the factors determining excretion of lead from the organism are practically the same as those determining liberation of lead from the bones. As lead is stored in the hard bone in man just as in animals (309), we therefore have employed upon patients suffering from lead poisoning the same methods which apparently proved effective in liberating stored lead in cats. The determination of the amount of lead eliminated in the excreta is, of course, but an indirect and inexact measurement of the quantity liberated from the bones, for lead may circulate through the body and be re-deposited without appearing

in either urine or feces. But the amount thus forming the "lead stream" in the blood is too minute to measure by any quantitative chemical methods now available.

In a special ward of three beds at the Massachusetts General Hospital, our patients have been under the charge of a special metabolism nurse who had no duties except to see that the necessary regime was carried out. Patients were usually transferred from the regular wards after the definite diagnosis of relatively severe lead poisoning had been established, and were promptly started on their special regime. All the excreta were collected; the urine in twenty-four-hour specimens (preserved by powdered thymol); the feces in individual specimens. Since chemical analyses were made of the excreta for three day periods, 0.3 gram of carmine alum lake was administered orally every third day to permit accurate collection of feces as well as urine. This dye was given at 1 P.M. and its first appearance in the feces marked the end of the period; the collection of urine for this same period ended at 6 P.M.

In order to do away with as many variable factors as possible during medication, the patients were given the same low calcium diet daily. During the first few days they were allowed to choose whatever seemed most attractive from the list of foods containing little or no calcium; and thereafter the daily ration was kept strictly uniform. The patients accepted this monotonous diet well. All food was weighed, and if any was not eaten its quantity was carefully determined and deducted from the total. A list of the different foods given and their calcium content follows:

Foods allowed in low calcium diets

	Content of calcium in per cent
Milk-free bread	0.011
Regular bread	0.041
Round steak	0.008
Liver	0.006
Chicken	0.011
Ham	0.022
Potato (raw)	0.011*
Macaroni	0.018
Rice (uncooked)	0.007
Rice (boiled)	0.006
Canned tomatoes	0.005
Canned corn	0.005
Apple, fresh	0.010*
Apple, dried	0.021

Peaches, dried	0.026
Bananas, fresh	0.007*
Butter fat	
Salt, pepper, sugar	
Coffee, tea	

* From Sherman.

The calcium intake was raised either by the addition of calcium lactate and milk to this uniform diet or by allowing the patient to receive the regular hospital diet plus a quart of milk and two grams of calcium lactate per day.

Under such uniform conditions, the factors influencing the excretion of lead, when absorption is no longer active, may be studied with comparative ease. Thus, sixty-five satisfactory observations (with an average duration of more than three weeks) have been made of the effects of various medications which influence the balance of salts, the calcium balance, or the acid-base equilibrium; or produce catharsis. Each new procedure was started with great caution in order to avoid precipitation of any such acute manifestation as colic. The influence of (*a*) magnesium sulphate, (*b*) potassium iodide, (*c*) potassium chloride, (*d*) high calcium intake, (*e*) low calcium intake, (*f*) diammonium acid phosphate, (*g*) phosphoric acid with both high and low calcium diet, (*h*) hydrochloric acid with high and low calcium diet, (*i*) ammonium chloride, (*j*) sodium bicarbonate, and (*k*) sodium citrate has been studied in twenty-seven of our cases—for the most part within the last two years. Symptoms of intoxication by lead appeared rarely during treatment; and at the time of discharge from the hospital all the patients had improved markedly—they had gained weight and were relieved of active symptoms. In three of the many patients treated a mild colic developed and in one encephalopathy recurred after administration of ammonium chloride, but this promptly disappeared with the restoration of a positive calcium balance.

As space does not permit a detailed outline of all the observations, some will merely be mentioned in a general way and the others will be reported in the summaries of the effects of medication.

Although before and after nearly every period of medication control observations were made, these could not always continue as long as was desirable, because of the difficulty of keeping men in the hospital for a pro-

TABLE 30

Control periods: consecutive observations of three-day periods without medication other than change in diet

DATE	DIET	LEAD EXCRETION			CALCIUM	
		Urine	Feces	Total	Intake	Output
	P. M.					
		mgm.	*mgm.*	*mgm.*	*grams*	*grams*
April 7 to April 28	Low calcium plus calcium lactate	0.52	0.25	0.77	2.172	2.350
	Low calcium plus calcium lactate	0	0.87	0.87	2.172	1.617
	Low calcium plus calcium lactate	0.44	0	0.44	2.172	2.661
	House diet	0.34	0.06	0.40		
	House diet	0.24	0.67	0.91		
	House diet	0.26	0.76	1.02		
	House diet	0.33	0.75	1.08		
May 19 to June 2	Low calcium diet	0.33	0.47	0.80	0.360	0.622
	Low calcium diet	0.33	1.22	1.55	0.408	0.711
	Low calcium diet	0.41	0.56	0.96	0.411	0.682
	Low calcium diet	0	0.80	0.80	0.411	1.450?
	Low calcium diet	0.26	1.57	1.83	0.411	0.868
June 24 to July 11	Low calcium diet	0.47	0.61	1.08	0.357	0.991
	Low calcium diet	0.46	1.30	1.76	0.357	0.707
	Low calcium then house diets	0.22	0.96	1.18		2.221
	House diet	0.10	0.69	0.79		4.467
	House diet	0.28	1.10	1.38		3.952
	House diet	0	0	0		3.282
	D. J. M.					
May 3 to May 18	House diet	0.43	0.52	0.95		4.500±
	House diet	0.72	0.33	1.05		2.600
	Low calcium diet	0.44	1.12+	1.56+	0.341	
	Low calcium diet	0	1.80	1.80	0.355	0.609
	Low calcium diet	0.43	0.74	1.17	0.355	0.756
June 3 to June 18	Low calcium diet after $NaHCO_3$	0.48	1.09	1.57	0.420	0.950
	Low calcium diet after $NaHCO_3$	0.26	1.30	1.56	0.420	0.937
	Low calcium diet after $NaHCO_3$	0.35	0.91	1.26	0.411	0.692
	Low calcium diet after $NaHCO_3$	0.35	1.21	1.66	0.405	0.897
	Low calcium diet after $NaHCO_3$	0.20	0	0.20	0.348	0.815
July 18 to August 4	House diet	0.69	0	0.69		2.249
	House diet	0	0	0		2.839
	House diet	0.22	0	0.22		
	House diet	0	0	0		
	House diet	0	0	0		
	House diet	0	0	0		

TABLE 30—*Continued*

DATE	DIET	LEAD EXCRETION			CALCIUM	
		Urine	Feces	Total	Intake	Output
J. T.						
		mgm.	*mgm.*	*mgm.*	*grams*	*grams*
April 7 to April 27	Weighed diet plus calcium lactate	0.39	0	0.39	2.172	2.720
	Weighed diet plus calcium lactate	0	0	0	2.172	2.765
	Weighed diet plus calcium lactate	0.32	1.43	1.75	2.172	3.118
	House diet	0.33	0.97	1.30		
	House diet	0.21	0	0.21		
	House diet	0.31	0.47	0.78		
	House diet	0.43	0.40	0.83		
May 19 to June 2	Low calcium diet after $NaHCO_3$	0	1.94	1.94	0.357	0.685
	Low calcium diet after $NaHCO_3$	0	1.38	1.38	0.405	0.731
	Low calcium diet after $NaHCO_3$	0.49	1.76	2.25	0.411	0.865
	Low calcium diet after $NaHCO_3$	0.43	0	0.43	0.411	
	Low calcium diet after $NaHCO_3$	0	1.30	1.30	0.411	1.109
June 27 to July 18	Low calcium diet	0.45	1.34	1.79	0.357	0.880
	Low calcium diet for two days	0.25	2.04	2.29		4.659
	House diet	0.31	0	0.31		10.998
	House diet	0.18	0	0.18		8.567
	House diet	0.13	0	0.13		9.045
	Low calcium diet	0.42	1.01	1.43	0.316	1.519
	Low calcium diet	0	2.34	2.34	0.399	1.566
M. R.						
July 3 to July 17	Milk, during severe lead colic	0.74	2.83	3.57		
	Milk	0	0	0		
	Milk	0.56	0.59	1.15		2.033
	Low calcium diet	0.22	0	0.22	0.252	0.794
	Low calcium diet	0	0.72	0.72	0.322	0.793
July 30 to August 10	House diet	0	0	0		
	House diet	0	0	0		
	House diet	0	0.37	0.37		
	House diet	0.09	0.79	0.88		

longed time. In several patients, however, it was possible to make a series of such observations which is of value as a control (table 30). These demonstrated considerable variation in which both diet and constipation played some part. As shown in animals, a diet deficient in calcium favors excretion, and this may also be seen in the three observations on the patient J. T., where the third period (June 27 to July 18) particularly shows a striking effect of change of diet. There is a possibility that the longer the deficiency in calcium the greater the effect of medication on the excretion of lead. Consequently several cases are reported in full (figs. 20, 21, and 22) to show the course of prolonged observations.

Individual variations in the control periods necessitate merely qualitative interpretation of the results of medication. As the number of observations is too small to permit statistical or mathematical study, and as diet and individual variations complicate interpretation greatly, only average figures will be given here as evidence of the effects of the different medications. Detailed data are reported in a separate paper (19).

Factors influencing excretion of lead. Administration of cathartics has long been the favorite treatment of lead intoxication. Croton oil, which was recommended by Tanquerel, has now been largely replaced by magnesium sulphate. The exact effect of these cathartics upon the rate of excretion of lead has not been previously determined. In table 31 are data obtained from three observations after daily administration of magnesium sulphate (in one case one-third ounce per day; in two cases one ounce per day). The average excretion of lead in nine three-day periods during ingestion of magnesium sulphate was 0.53 mgm.; that in five control periods was 0.30 mgm. Thus, although it has a marked cathartic action, magnesium sulphate does not greatly influence the total lead excretion. It does, however, cause improvement in cases of lead colic, probably by relieving constipation and cleansing the gastro-intestinal tract, as well as by relieving spasm of smooth muscle, and not by increasing the rate of elimination of lead.

Potassium iodide. Quite another type of medication which has been employed for many years is the administration of potassium iodide. Melsens (297), who recommended its use in 1840, carried out experiments on dogs which have been supplemented by further

investigations by Parkes (352), Pouchet (366), and Dixon Mann (279) (see page 210). Their results are contradictory. Parkes and Pouchet believed that potassium iodide increased excretion, but Mann could not observe that it had any such effect. As no definite conclusion had been drawn when our investigation was started, one of the first problems was to determine its efficiency. Analysis of the

TABLE 31

The influence of medication

The average total excretion of lead for three-day periods

MEDICATION	NUMBER OF CASES	CONTROL PERIODS		PERIODS DURING MEDICATION		FIRST PERIOD AFTER MEDICATION		SUBSEQUENT CONTROLS		RATIO: AVERAGE VALUE DURING TREATMENT / AVERAGE VALUE BEFORE TREATMENT
		Number of periods	Lead excreted	Number of periods	Lead excreted	Number of periods	Lead excreted	Number of periods	Lead excreted	
			mgm.		*mgm.*		*mgm.*		*mgm.*	
Phosphoric acid with low calcium diet	10	19	0.81	45	2.51	9	0.92	15	0.53	3.1
Phosphoric acid with normal diet	12	22	0.46	43	1.10	7	1.54*	2	0.42	2.4
Hydrochloric acid	4	7	0.31	13	0.88	2	0.42	1	0	2.8
Ammonium phosphate	2	5	1.27	11	1.60	1	0.36	1	0.95	
Ammonium chloride	8	17	0.80	41	2.35	6	1.17	14	0.90	2.9
Tartaric acid	2	1	0.63	7	0.52	2	0.69	1	1.37	
Lactic acid	2	4	0.60	5	0.54					
Sodium bicarbonate	7	17	0.71	34	1.93	5	1.55	15	1.15	2.7
Sodium citrate	1	2	1.51	5	1.72					
Potassium iodide	9	10	0.68	28	1.29	6	1.19	8	0.50	1.9
Ammonium iodide	1	2	0.87	4	2.34					
Potassium chloride	2	2	0.57	7	0.57	1	0.53			
Magnesium sulphate	3	3	0.41	9	0.53	2	0.14			

* Greatly elevated by one high figure of 5.69 mgm.

excreta both before and during the use of potassium iodide clearly demonstrated (see table 31 and fig. 20) that elimination of lead increases during ingestion of this drug. When no lead was being excreted without treatment, potassium iodide caused its appearance in the excreta, and in those cases in which lead was present during the control periods, the total excretion was doubled—an average

excretion of 0.68 mgm. (ten periods) rose to 1.29 mgm. (28 periods). This increase continued during the first period after potassium iodide was discontinued.

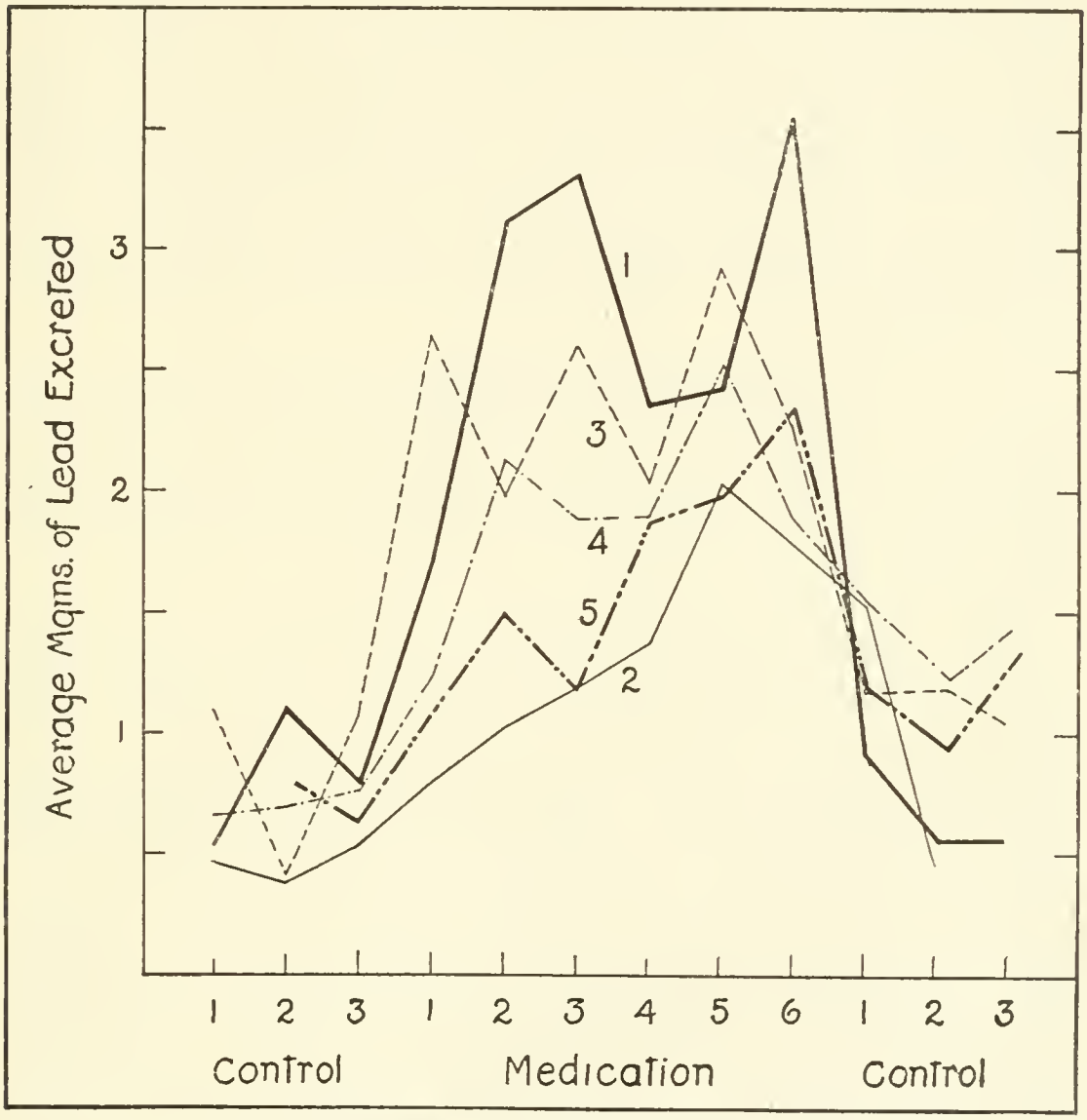


FIG. 20. SUMMARY OF EFFECTS OF MEDICATION

Each curve represents the average excretion of lead for consecutive three day periods.

Curve 1. The effect of phosphoric acid and a low calcium diet. The first control represents only two observations.

Curve 2. The effect of phosphoric acid and a normal diet. The first and last control periods represent only two observations each.

Curve 3. The effect of ammonium chloride.

Curve 4. The effect of sodium bicarbonate. The last medication period represents only two observations.

Curve 5. The effect of potassium iodide. The fourth, fifth, and sixth medication periods, the high points in the curve, represent only single observations.

A few experiments were performed in an attempt to determine whether it was the potassium or the iodide ion which produced this effect. Potassium chloride was administered in three experiments (5 grams daily) without obvious change in rate of excretion. The action of ammonium iodide was then tested because in the body ammonia would largely form urea which should exert no effect (see also ammonium phosphate experiments). Our one observation indicated that the definite increase of lead excretion was hardly less marked than with the potassium iodide which followed (see table 31). These experiments therefore suggest that it is the iodide ion which influences lead excretion.

Although potassium iodide has long been used empirically with fair success in the attempt to increase excretion of lead, its mode of action is unknown. The determination of various factors involved in the mobilization of lead, and therefore of effective methods for its control, has seemed desirable. Therefore, the medications tested on animals were further studied in man.

Acids. The effect of acids must be dependent upon (*a*) their toxicity and (*b*) their destruction in the body. The toleration of the organism to them apparently varies with the strength of the acid. Far larger quantities of phosphoric than of hydrochloric acid may be taken without discomfort. Ammonium salts, however, which Haldane (183) has shown to be effective in producing acidosis, may be administered with far more ease than any of the acids. Although *in vitro* certain acids dissolve lead more readily than others in equivalent concentration (hydrochloric, phosphoric, lactic, and tartaric acids, in order of increasing solvent action), it is not to be expected that this would be true in the body, for in the organism acids must be neutralised or be burned. The organic acids which are burned must have less effect on the hydrogen ion concentration of the organism and on the solubility of lead unless they are locally produced. Lactic or citric acid, for instance, when taken by mouth should theoretically be ineffective.

In treating patients in the hospital, results were obtained which seemed to bear out these differences. Daily administration of about 140 cc. of N/10 hydrochloric acid in four cases raised the average excretion from the control level of 0.31 to 0.88 mgm. per period, and

the rate of excretion fell to an average of 0.28 mgm. after the acid was stopped (see fig. 20 and table 31). These were early experiments, however, and were not as satisfactory as later observations.

Phosphoric acid was administered with diets of both high and low calcium content. Twelve observations were made with high and ten with low calcium intake. During most of the control periods the diet contained much calcium. In nearly all of the individual experiments the excretion of lead increased during the ingestion of acid. Figure 20 demonstrates the average curves obtained from all the observations.[1] The striking increase in excretion of lead in both series is shown in

TABLE 32

The daily urinary excretion of phosphorus

	PATIENT		
	H.S.	P.C.	B.L.
	gm.	*gm.*	*gm.*
Before acid	1.47	1.20	0.47
	1.53	1.25	0.49
	1.58	1.00	0.36
			0.73
During ingestion of phosphoric acid	3.47	2.52	2.08
	3.16	2.46	2.04
	2.26	2.84	2.05
		2.36	1.88

table 31. Apparently acid with a low calcium diet caused a greater excretion of lead than occurred when the diet was normal. This is more striking than the chart shows, for the average control figure is elevated because eight of the nineteen control observations were made during a low calcium intake. In these, the average excretion per period was 1.27 mgm. as compared with that of 0.48 mgm. during the eleven control periods when the diet was rich in calcium.

That the phosphoric acid was absorbed by the gastro-intestinal tract was determined in several ways. The quantity of inorganic phosphate in the urine when the diet was constant proved much

[1] These curves do not represent the same number of experiments in each period, because all observations were not equally prolonged. Accurate comparisons can best be made by reference to the data of each observation which is given in our original paper.

greater than normal (see table 32) and the increase could only have been derived from the acid absorbed. Determinations showed that the quantity of urinary ammonia gradually rose to a high level during acid ingestion. The CO_2 combining power of the blood plasma was definitely reduced during ingestion of acid in a majority of our numerous determinations. Dr. A. O. Koehler has also demonstrated that the hydrogen ion concentration of the blood fell during acid ingestion to a figure as low as 7.19. As soon as administration of acid ceased, these figures returned promptly to their previous normal values.

In an effort to produce acidosis without the discomfort of direct ingestion of acid, the ammonium salts of both phosphoric and hydrochloric acids were administered (183). Ammonium phosphate ($(NH_4)_2HPO_4$) proved rather ineffective in two cases. It increased the average excretion from 1.27 mgm. per three-day period before medication to 1.60 mgm., but the average excretion fell to 0.66 mgm. of lead per period after medication ceased. Ammonium chloride, on the other hand, when administered with a diet of low calcium content, gave much more satisfactory results (eight cases), in fact it appeared to be as efficient an agent as phosphoric acid. The daily dosage varied somewhat, but could be as large as 12 grams. Just as during treatment with phosphoric acid, the hydrogen ion concentration of the blood plasma fell and the CO_2 combining power was diminished while this salt was being ingested. Increased quantities of ammonia gradually appeared in the urine under this regime. Thus it is demonstrated that ammonium chloride produces acidosis quite comparable to that caused by phosphoric acid.

Alkalies. Because of the greater solubility of tri-lead phosphate in alkaline as well as acid medium, attempts were also made to increase elimination of lead by rendering the tissues more alkaline than normal. Sodium bicarbonate (in doses up to 40 grams per day) given with the usual varied diet produced striking results in seven cases (table 31 and fig. 20). It reduced the urinary excretion of nitrogen, increased the CO_2 combining power of the blood plasma, and changed the hydrogen ion concentration to pH 7.55. Although sodium bicarbonate does not cause as great an increase in the rate of elimination of lead as do the acids or acid-forming substances, it possesses certain advantages, for it was given without a special diet and seems to produce

an effect which continues longer after cessation of treatment than that caused by other forms of medication. In one observation sodium citrate was found to increase only slightly the rate of excretion (see table 31).

As all of these drugs were ingested in quantities which approached the limit of tolerance, it was necessary to watch the general condition of patients with care. Usually they lost appetite, and during the development of acidosis sometimes suffered from headache and general malaise. The weight which was lost during treatment with acids was rapidly regained during control or rest periods. Ammonium chloride and sodium bicarbonate seemed to have less general effect. Occasionally medication produced diarrhea. In no case, however, was it the cause of an acute manifestation of poisoning; and in patients suffering from wrist drop satisfactory improvement progressed. Under the regimes thus outlined, recovery from anemia was rapid, and the stippling of red blood cells gradually disappeared. The general condition of the patients was excellent and they regained strength, gained weight, and recovered good color.

In order to give a clearer picture of our results, the charts of three patients are included. These show the effect of diet and medication upon the excretion of lead for a long time and include only the more important data. Such various factors as calories or type of food, salt intake, and volume of excretion, which apparently exert but little if any influence on the lead excretion, are omitted. The longer observations were made on individuals who, because they were temporarily disabled by bilateral wrist drop, could be kept in the hospital indefinitely.

In looking over the charts it is important to observe not only the effect of the medication but also that exerted by the calcium intake and balance.

M. G. H. 259852. John L., thirty-two, Austrian, chauffeur.

Nine days before entrance he had a chill, fever, and pain in joints, with persistent constipation. Two days ago he developed severe generalized abdominal cramps. This is his second such attack.

Physical examination showed a pale man with a marked lead line. The abdomen is soft but tender in epigastrium, and the liver edge is palpable and tender. There is no muscle weakness.

Laboratory findings: Red blood cells 3,600,000. Hemoglobin 65 per cent. White count 8000. The reticulated red cells were 3 to 6 per cent, and many stippled cells were found. The serum dilution of his blood was elevated to a level of 1:40, and bile obtained by duodenal tube showed increased amount of bilirubin, both of which suggested increased blood destruction. X-rays of lungs, gall bladder and gastro-intestinal tract were not remarkable. Blood pressure 140/70.

Course: He continued to complain of some right upper quadrant pain until it was definitely decided that his gall bladder was normal. The source of the lead was not discovered. He left the hospital feeling well, having gained six pounds.

The treatment and lead excretion is shown in fig. 21.

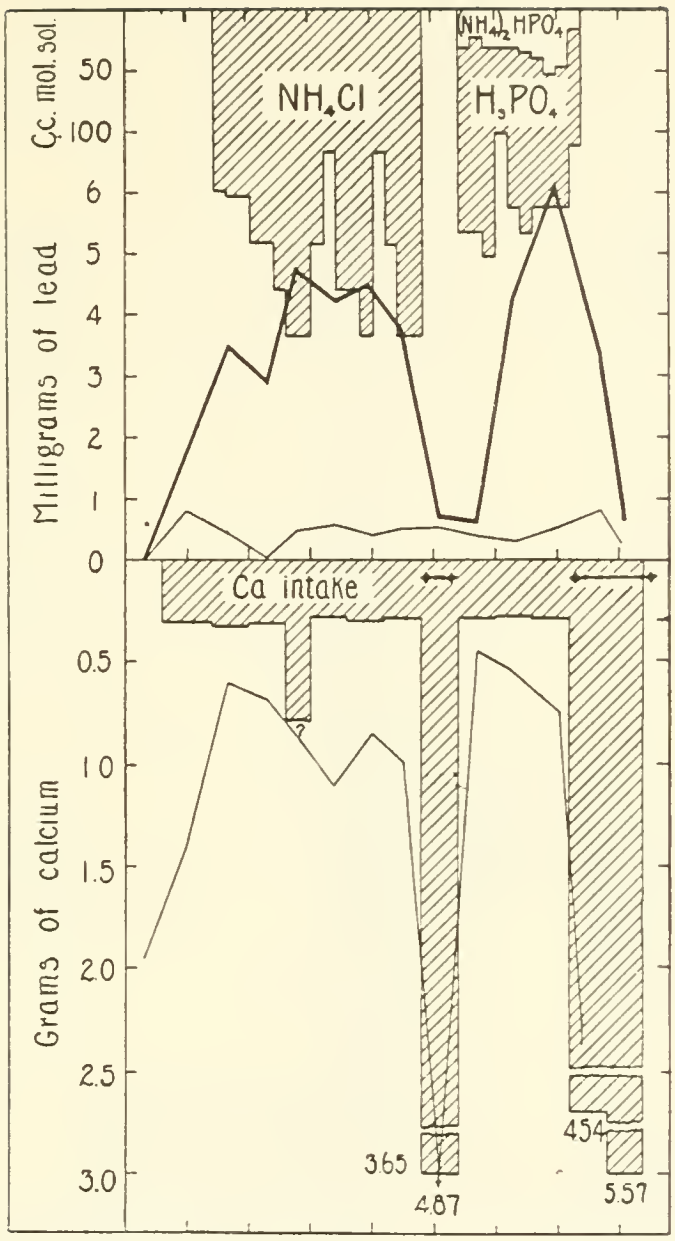
M. G. H. 260749. John T., thirty-four, Lithuanian, rubber mixer for five years.

Three months ago he developed severe colic with marked constipation and vomiting. Then he noticed weakness of wrists, which was most marked in the morning. He worked until three days ago, when he was too weak to continue. Suddenly he became maniacal and had a convulsion and was taken to the Psychopathic Hospital. He was pale, wildly maniacal, showed marked confusion, was completely disoriented and violent, and had hallucinations of sight and smell.

Physical examination showed marked pallor and lead line. He had a bilateral wrist drop with involvement of upper arms of the brachial type of Remak, involving deltoid and supraspinatus muscles on the right and left, though palsy of deltoid and supraspinatus muscles on the left was not complete. There is definite swelling in the tendon sheaths of the paralyzed wrist muscles.

Laboratory findings: Red blood count 3,800,000 with marked stippling of blood cells. Spinal fluid was normal, except for increased pressure, though lead was found in it. The Wassermann test was negative in blood and spinal fluid. The renal function, non-protein nitrogen, blood calcium, phosphorus and sodium chloride were all normal.

Course: Patient recovered completely from mania within twenty-four hours after high calcium intake was started. In three weeks he was transferred to the Massachusetts General Hospital, and was kept for a long time because he was economically completely disabled. Gradually strength returned in his hands and arms, and at discharge (after seven months) there was good movement in all muscles though the extensors of the wrist



FIGS. 21, 22 AND 23. THE EFFECTS OF MEDICATION AND DIET UPON LEAD EXCRETION IN INDIVIDUAL CASES

The interval marks on the abscissa represent five days

Above the middle horizontal line is shown the medication and lead excretion. The faint line is the excretion of lead in the urine expressed in output for three days. The heavy line is the total excretion of lead in the urine and feces for three day periods. The medicaton is shown in blocks representing the cubic centimeter of molar solution taken per day. These really represent as much as the patient could take without toxic symptoms.

Below the middle horizontal line is shown the calcium intake in blocks of three days total ingestion. The arrows in areas of high calcium intake represent the addition of milk or calcium lactate to the routine low calcium diet. The areas which have no enclosing block line represent periods of unweighed full diets. The single line represents the total calcium output in urine and feces in three day periods. The distance between line and blocks therefore represents the positive or negative calcium balance.

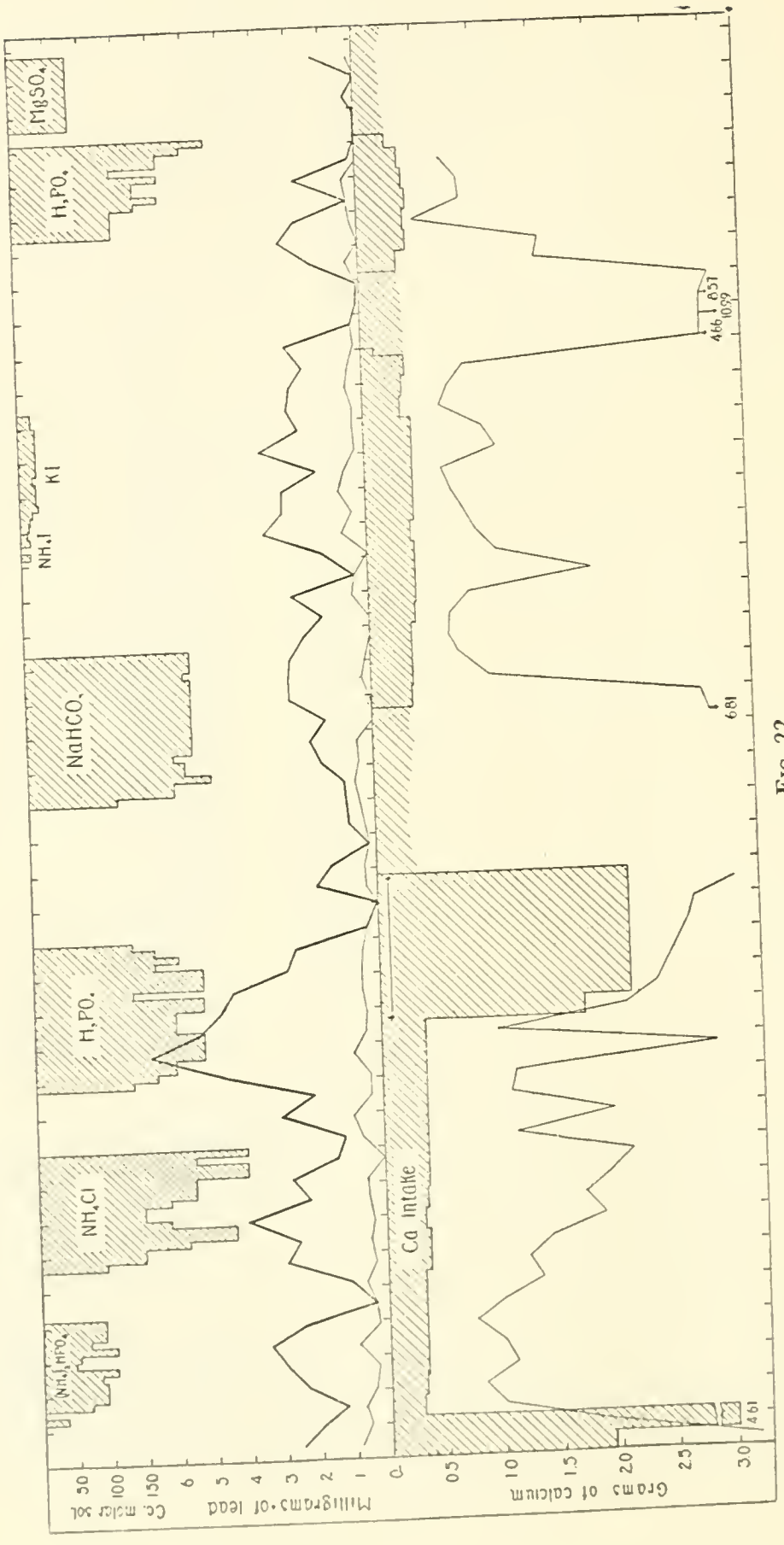

MgSO4
H3PO4
KI
NH4I
NaHCO3
H3PO4
NH4Cl
Ca intake
681
466
1,651
1059
461
Cc. molar sol
Milligrams of lead
Grams of calcium

FIG. 22

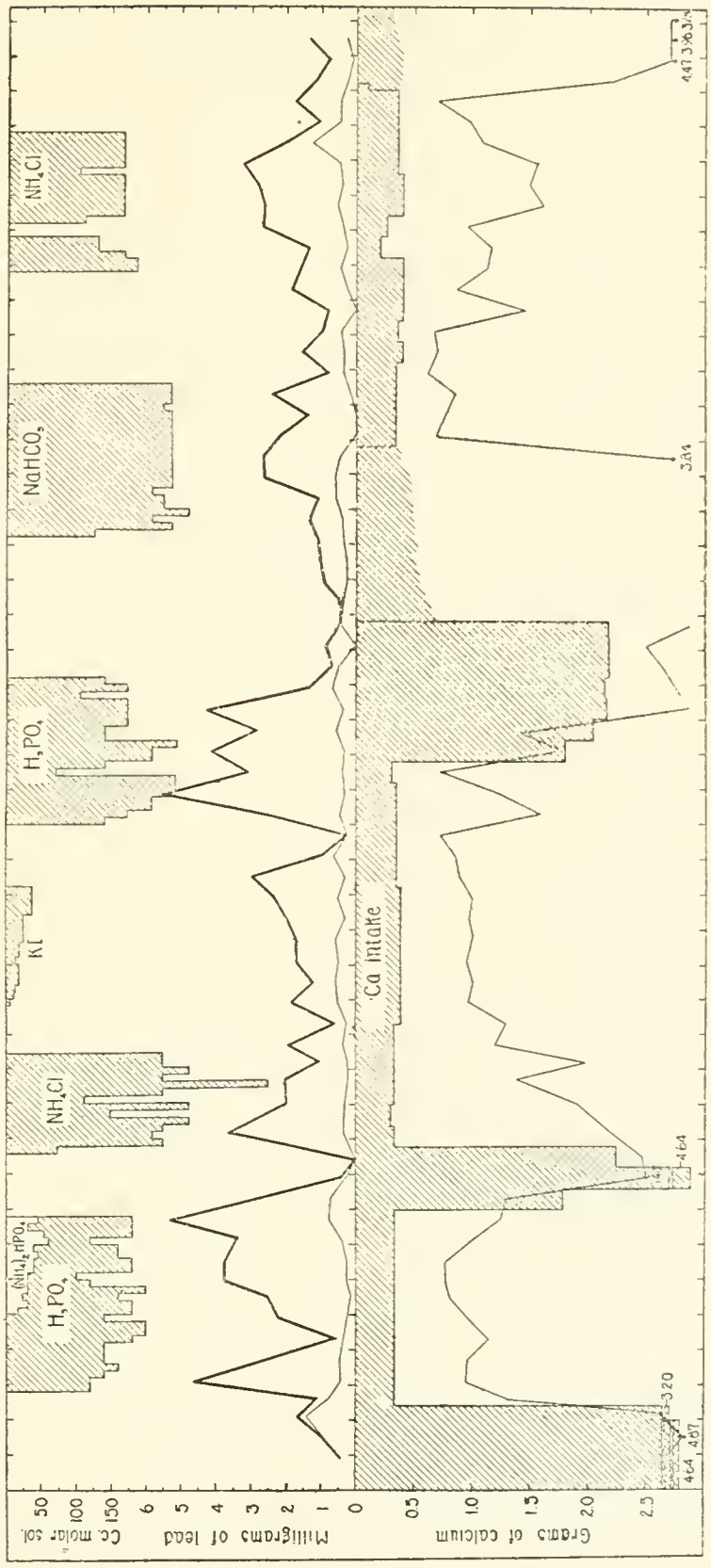

Cc. molar sol.
Milligrams of lead
Grams of calcium
Ca intake
H3PO4
(NH4)2HPO4
NH4Cl
KI
H3PO4
NaHCO3
NH4Cl

FIG. 23

were still not strong. During his 204 days in the general hospital he felt well, complained of no colic, and gained twenty pounds in weight.

His treatment and lead excretion are shown in figure 22.

M. G. H. 259296. Pando M., forty-eight, Greek, rubber mixer for seven years.

For five months he has had repeated attacks of severe abdominal colic. For four months he has felt "burning" sensations of feet and easy fatigue. Seven weeks ago his hands and arms felt "burning," and his hands became weak. The ring fingers were first involved, then the middle, and later the index finger, and finally the wrist. The palsy in the right hand was more severe than the left.

Physical examination showed a pale, wan-looking man with a marked lead line. His arms showed no sensory disturbances, but great weakness in all movements, particularly in biceps and deltoid, and there was a typical double wrist drop which was more marked on the right. Both legs were weak and there was a slight but definite toe drop on the right. There is definite swelling in the tendon sheaths of the wrist muscles which are paralyzed.

Laboratory findings: Hemoglobin 75 per cent. Red blood cell count 3,700,000, with marked stippling of the cells. Non-protein nitrogen of blood was 51 mgm. per cubic centimeter, but the calcium, phosphorus, and chlorides were normal and the sulphone phenolphthalein excretion averaged 50 per cent in two hours.

Course: Colic stopped promptly on entrance, and in two months his upper arms had markedly regained strength. The wrist drop gradually improved, and at discharge, both hands were still weak, but all movements could be made. He felt well except for continued burning sensations in his legs. The red cell count was 4,900,000 and there was no stippling found. He gained twenty-two pounds during his 237 days in the hospital.

His treatment and lead excretion are shown in figure 23.

Summary. The various observations on both cats and man seem to demonstrate that both acids, acid-forming salts, and alkalies definitely increase the excretion of lead to a greater extent than does potassium iodide. Magnesium sulphate has apparently no such effect. Although acids increase excretion of lead when the diet is normal, they are much more effective when the calcium intake is low. Sodium bicarbonate and potassium iodide produce their action without a

special diet. In potassium iodide it is apparently the iodide ion which is effective.

Part II. Pathology

X. PATHOLOGY

The lack of correlation between the clinical picture of lead poisoning and the pathological findings has produced much controversy. Patients who have presented many symptoms suggestive of pathological change usually show at post mortem examinations little of interest. Moreover, the many ante mortem symptoms and signs are explained by the autopsy with great difficulty and lead is rarely proved to be the immediate cause of death (3). Under such conditions the confusion in the literature may readily be imagined. Not only have many opinions been based on the examination of isolated cases, but incorrect conclusions have been drawn from autopsies which revealed lesions characteristic of conditions other than lead poisoning. Facts such as these probably led conservative and careful clinicians to sound a warning that appendicitis as well as colic can attack a lead worker and that those who have absorbed lead are not immune to cerebro-spinal syphilis.

Even experimental lead poisoning has done little to clarify the situation. Many of the investigations have been uncontrolled and certain of the pathological conditions attributed to the action of lead may frequently be seen in supposedly normal animals. No doubt some work suffered from attempts to demonstrate a relation between the lesions found in an organ and the quantity of lead recovered from it. This, of course, led to the accumulation of much contradictory evidence and complicated the question by a fruitless discussion as to whether lead acts directly or indirectly. Some investigators believed that lead so affected the organism that an abnormal metabolism resulted and permitted the formation of toxic substances.

Since our patients have shown little that can be definitely ascribed to the action of lead, and since microscopic examinations of the tissues from our animals have not yet been made, the main purpose of the following account is to review the literature in so far as it in-

cludes observations which have been made with some regularity, and have been confirmed, or which have an obviously important bearing on the subject. In addition, in order to avoid increasing the existing confusion, an attempt will be made to evaluate these observations so that some idea may be obtained of the relative importance of the lesions attributed to lead.

Students have always endeavored to crystallize into a pathological entity the isolated observations of each clinical condition. To this end wherever possible they have sought to attribute a specific lesion to each etiological factor. Lead has been no exception.

The specificity of the lesion in lead poisoning has been most strenuously advocated by Legge, Goadby, and Goodbody (252) (166). These workers claim that the specific lesion in lead poisoning consists of hemorrhages caused by the action of lead on the vessels, especially the minute venules. Following this condition secondary pathological changes may occur, particularly fibrosis, as was suggested previously by Siccardi (430) (431) and also Hitzig (218). This idea of a characteristic change in plumbism was based on results in some animal experiments and more particularly on a case reported by Mott (321). There are many objections to this view. One of the most frequent signs of lead poisoning is anemia, often of marked degree (section XVII). This alone is sufficient to explain the occurrence of hemorrhages. Moreover, Mott's patient had nephritis and arteriosclerosis, conditions frequently associated with hemorrhages. Mott definitely states that many of the lesions were probably artefacts. Catalano (72) also believed that hemorrhages constantly accompanied lead poisoning; but his patient suffered from convulsions, and there is no reason to believe that this symptom may not have been a contributing cause rather than the result of hemorrhage. Siccardi performed experiments on isolated arteries, perfusing them with salts of lead. He found that these vessels shrank markedly and concluded that lead acts on the smooth muscle of the media. These tests, however, were performed entirely *in vitro* and with very concentrated solutions of lead. Their applicability, therefore, to conditions *in vivo* is not established. In sections of liver, which we perfused with lead salts, the lead was entirely taken up by the endothelial cells of the vessels, none penetrating into the media. The evidence offered for the theory of vascular injury, however, has not changed the opinion of most observers, which holds that there is no lesion characteristic of lead poisoning, except perhaps the lead line.

General appearance. At post mortem examination, patients who have had lead poisoning present the appearance of marked undernourishment which is emphasized by absence of subcutaneous fat.

Mucosa. Probably the most characteristic and common finding is the so-called lead or Burtonian line—a dark line on the gingiva near the border of decaying teeth. This is not a superficial formation, for it cannot be rubbed off. Microscopic examination of small sections snipped from the gums shows that the lead line is composed of fine black amorphous granules which lie in the connective tissue at the base of the epithelium. These are also found in the interior and in the walls of vessels, in connective tissue fixed cells, and in macrophages. A similar black discoloration in the mucosa of the colon and in the lower portion of the small intestine, first observed by Tanquerel (453), is sometimes seen.

Gastro-intestinal tract. The common occurrence of colic in plumbism has focused the attention of pathologists on the gastro-intestinal tract and several lesions have been reported.

In acute fatal cases, Hutton (222) states that entero-colitis results from the caustic action of lead; while in chronic cases a long-continued gastro-enteritis, consisting of glandular atrophy of the stomach and small intestine, and intertubular diffuse sclerosis, has been frequently observed (299) (243). Legge and Goadby have noted a slight degeneration of the muscular coats with infiltration and minute hemorrhages. Maier (274), who also reported finding hemorrhages in the gastro-intestinal tract, contributed to this list of changes a fatty degeneration of secretory cells, enlargement of arteries, venous stasis, and circumscribed areas of softening. Of special clinical interest are the experiments of Jores (230) in which he produced gastric and intestinal ulcers in dogs by the administration of lead. These are significant because of the frequent occurrence of ulcers in lead intoxication, and may suggest a method of investigating the mechanism by which such lesions are formed.

Various pathological changes have been reported as the underlying cause of colic. Maier believed that sclerotic degeneration of the submucous and myenteric plexuses is the fundamental factor in its etiology. This abnormal condition of the sympathetic innervation of the gastro-intestinal tract has also been reported by Tanquerel who described hypertrophy of the coeliac ganglion, and by Mosse (320) who thought that sclerosis of the sympathetic ganglion was always the cause of colic. Both Tanquerel and

Oliver (337) have reported that the intestines are contracted when colic has occurred before death; but no description of the lesion actually responsible for this condition has ever been given. Alcock, moreover, says that he has never observed the contraction in human autopsies, although he frequently found it in his guinea pigs. Meillère (293) believed that lead may cause a general hypertrophy of all glandular appendages of the gastrointestinal tract followed by atrophy which gives the senile appearance to the tissues; but he emphasized that most of the lesions described by investigators might very well be due to alcohol and that their significance in the diagnosis of lead poisoning should be questioned.

Liver. The rôle played by lead in the production of hepatic lesions is of considerable interest because of the importance of the liver as an eliminator of lead, especially of lead which enters the system by way of the mouth, and because of the supposed relationship of lead to the production of cirrhosis of the liver.

Several types of lesions of precirrhotic or cirrhotic character have been described. Hutton, Potain (364), and Alcock have reported the occurrence of atrophy of the liver cells, fatty degeneration or infiltration, and intercellular or interlobar cirrhosis; and Legge and Goadby add that small areas of exudation and hemorrhage may also be seen. Kussmaul and Maier believed that lead acts directly on the portal vessels, but Hanot and Charpentier (197) thought that the hepatic cells themselves were directly affected. Meillère states that periportal cirrhosis may be produced experimentally by lead, but Ophüls (340), who worked with guinea pigs, never saw this lesion. In his animals a marked necrosis of cells was followed by a collapse of the tissue and dilatation of veins, which he called "chronic focal atrophy." To this he never attached much importance, for he believed that the only condition attributable to the action of lead was hematogenous pigmentation of the organ. With very large doses of lead acetate Mallory (277) produced basophilic instead of acidophilic hyaline lesions within the hepatic cells, necrosis and phagocytosis of these cells, and saw evidences of regeneration in the mitotic figures within some of them. Striking definite cases like that reported by Seeligmüller (412) are rare. He found evidences of hepatic cirrhosis in the liver of the new-born child of a lead worker and recovered lead from the organ.

The relationship of lead to so-called alcoholic cirrhosis has been the subject of much controversy. It is a far cry from Chauffard's

(78) belief that all cirrhosis of the liver in plumbism is really caused by over-use of alcohol, to Mallory's statement that all cirrhosis attributed to alcohol may be due to contaminating lead. In spite of all the pathological conditions which have been noted in plumbism, the consensus of opinion among pathologists who have studied lead poisoning confirms Oliver's view that post mortem examination of the liver reveals no changes specific for lead.

Heart. The heart, as might be expected from clinical observation, shows nothing remarkable as a result of lead intoxication. Such cardiac lesions as hypertrophy, secondary to supposed vascular and renal changes (347), myocardial degeneration following hemorrhage (252), and even brown atrophy (243) have been described, but most observers believe that the heart undergoes no real change, certainly none due to the direct action of lead (337).

Vascular system. With respect to the effect of lead on the rest of the vascular system, however, there is much less agreement.

As previously mentioned, the blood vessels have been considered the principal point of attack of lead chiefly because of the work of Legge and Goadby, who described the occurrence of perivenule hemorrhages. But even before these investigators presented their view, Henle (208), Heubel (213), Maier (274), Hitzig (218), Rosenstein (390), Elschnig (114), and others believed that the vascular system was first involved. Elschnig thought he could demonstrate an actual degeneration of the unstriped muscle of the vessels. Even arteritis, sometimes to an obliterating degree, has been reported (463). In this connection it is of interest to note the case cited by Kazda (232) of a patient with gangrene of the legs supposed to be due to lead arteritis. Despite all this evidence and the fact that Gouget (175) observed aortitis in one of his guinea pigs, it is noteworthy that Ophüls never was able to produce vascular lesions experimentally. Although the general clinical opinion holds that arteriosclerosis is a result of chronic plumbism, this idea is based entirely on the occurrence of this condition in many post mortem examinations. That arteriosclerosis is much more frequent in those exposed to lead than in other individuals of the same age, however, needs much more statistical proof before it can be accepted.

Kidney. The effect of lead on the kidney is of very great practical importance both with respect to nephritic conditions and also because of the possible uremic origin of encephalopathy. Two types

of lesions have been reported, an acute and a chronic nephritis. Oliver describes an acute tubular nephritis characterized by cloudy swelling and fatty degeneration of the tubules with destruction of the cells. Occasionally cellular proliferation in glomeruli appeared with leucocytes gathered around the afferent vessels. In some cases (222) an atrophy of the glomeruli with hyaline degeneration of the vessels has been seen. The chronic nephritis supposed to be caused by lead presents the picture of the typical secondarily contracted kidney.

Charcot and Gombault (76) think the lesion is primarily an epithelial cirrhosis. On the other hand, Legge and Goadby maintain that such a condition in lead workers has a vascular origin, but suggest that kidney changes have never been directly traced to lead and are probably due to alcohol. Such a stand is of interest in view of the fact that Oliver, who is so conservative in his conclusions, states quite definitely that in chronic plumbism chronic interstitial nephritis with small contracted kidneys and thickened arterioles is a frequent finding.

Experimental work has not yet succeeded in deciding just what part lead plays in establishing nephritic conditions. Experiments were carried out by Ophüls who used guinea pigs in an effort to produce renal lesions. He found pathological changes in the distal convoluted tubules, consisting of granular degeneration, pyknotic nuclei and necrotic cells. There were also many epithelial casts and some evidence of regeneration of the epithelium. However, he very significantly points out that this entire picture was frequently seen in his control animals. He therefore holds that the only constant, but very limited, lesion is some degeneration and fibrous thickening of the glomeruli which he did not see in the normal guinea pigs.

The only attempt to study "lead nephritis" statistically has been made by Machwitz and Rosenberg (272). They investigated the relative incidence of Bright's disease in lead workers compared to other laborers and found that of thirty-six cases of "malignant sclerosis" seventeen occurred in laborers, nine of whom had worked with lead. They also quote Volhard who reported that four of his thirty-six patients with Bright's disease were lead workers. These are the only available statistical reports of the incidence of nephritis in lead workers and because the figures are so scanty no definite conclusion can be drawn from them. Here, as in the case of arteriosclerosis, the accepted clinical opinion that lead can cause chronic interstitial nephritis is far from convincing. Certainly the urinary, blood pres-

sure, and blood chemistry findings hardly point to a marked kidney involvement. This is all the more interesting because of urinary excretion of lead.

Brain. The severity of lead encephalopathy with its uncontrollable symptoms and high mortality makes the question of the action of lead on the nervous system of more than passing interest. A full discussion of the possible pathogenesis of encephalopathy will be given in section XVI, but in this section only the different pathological pictures described by various observers will be reviewed.

The brain has been seen by various workers to be shrunken, firm, pale, sometimes edematous, often icteric, and the convolutions may be flattened or atrophied (453) (293) (337). Considerable attention has been paid to the microscopic picture of the brain in encephalopathy. Hutton mentions that arteriosclerosis and arteritis may be found; and Mott, Legge and Goadby, and Courtney (85) have observed disseminated hemorrhages in the perivascular spaces after chronic encephalitis. They thought these were caused by arterial and venous degeneration, and considered them the characteristic lesion of this condition. Mott, in his case, saw marked hyperplasia of the neuroglial cells. Round cell infiltration has also been noted, but frequently when the picture is complicated by syphilis, as in two of our cases.

Perhaps the most important contribution to this subject has been the examination of the cerebro-spinal fluid of encephalopathic patients by Mosny and Malloizel (316), Norton (332), and Boveri (50).

These investigators report a constant pleocytosis (approximately 100 lymphocytes per cubic millimeter cerebro-spinal fluid), an increased quantity of globulin, and increased pressure. Hassin's (203) careful pathological examination of brains after uncomplicated lead encephalopathy gave results which fit in well with these observations. He saw marked proliferation of fibrous tissue and blood vessels in the pia-arachnoid space, especially near the cerebellum, optic chiasma, and temporal lobe; and infiltration by lymphocytes and plasma cells in the relatively acute cases. The parenchyma was only very slightly affected, and was described by Hassin as follows: "The glia cells, like the pia-arachnoid, showed marked progressive changes; increased size of nuclei, abundance of chromatin and cytoplasmic processes, and a great proliferation of glia nuclei gathered in rows or clusters.

They usually contained fat and frequently invaded the ganglion cells. Most of the latter were well preserved, some showing chromatolytic changes, vacuolation and neuronphagic phenomena." He found that although this proliferation of blood vessels was most marked in the meninges and choroid plexus, it occurred to some extent in the cord, cerebellum, mid-brain, cortex, subcortical white substance, and large ganglia, and was not only an endothelial and adventitial overgrowth, but also a formation of new capillaries. Signs of perivascular infiltration were, however, strikingly absent. After a consideration of all these microscopic findings Hassin concluded that lead encephalopathy contrasts sharply with infiltrative, infectious encephalitis. In plumbism the meninges, the base of the brain, the cerebellum, the optic chiasma, and the temporal lobe are involved, while epidemic encephalitis affects chiefly the mid-brain. In his opinion round cell infiltration in lead encephalitis is merely the manifestation of a reaction against the irritating substance, and is characteristic of the more acute form of the disturbance. It is interesting that Hassin makes no mention of the hemorrhages which play so large a part in many neuropathological descriptions of lead encephalopathy.

The question, therefore, arises whether lead encephalitis is not really a meningo-encephalopathy, which is primarily a chronic productive meningitis, characterized during acute exacerbations by a degree of round cell infiltration varying with the severity of the attack. Certainly most of the recent pathological and clinical pathological observations seem to bear out this theory.

Spinal cord. The occurrence of a type of amyotrophic lateral sclerosis in lead poisoning makes a study of the pathology of the spinal cord of value. Gross changes have never been observed (453), but many microscopic alterations have been found. A change most constantly seen and most closely related to the clinical picture of muscular atrophy is the degeneration of the anterior horn cells (210) (443) (293) (321). This is characterized by vacuolation of the ganglion cells, perinuclear chromatolysis, fatty pigmentation, and marked shrinking of the cells.

Hutton reports that following degeneration of nerve fibers there is an increased neurogliosis and proliferation of connective tissue. Catalano, and Legge and Goadby describe the occurrence of punctate hemorrhages within the cord also; and Hitzig states that inflammation of blood vessels

is an important and common lesion. A lymphocytic infiltration around the adventitia of the arteries has been reported by Mott; and some observers have found that distention and actual alterations of the venules may be seen. The older investigators believed that nuclear proliferation occurred frequently (313) (406) (490).

In cases of atrophic paralysis, therefore, the lesion is probably amyotrophic in character and may perhaps include some sclerosis of the lateral columns.

Peripheral nerves. Perhaps no question in the pathology of lead poisoning has been the subject of so much controversy as the site of the action of lead in palsy. Although typical lead paralysis is not of the atrophic type just described, but resembles so-called peripheral neuritis, it has been attributed by some authors to cord lesions. However, the consensus of opinion seems to confirm the peripheral location of the lesion and is based upon the observation of very distinct changes in the peripheral nerves themselves.

The older writers, especially von Monokow, Schultze, Westphal, and Eichhorst (108), were impressed with nuclear proliferation especially around the vessels. Eichhorst also believed that the blood vessels were thickened and thought, as did Meillère and Déjérine-Klumpke (92), that the essential lesion was a homogenous degeneration of the sheath of Schwann—a typical Wallerian degeneration. Déjérine-Klumpke, quoting Gombault (171), emphasizes that the lesion is essentially peri-axial in character, and that the axis cylinders themselves are not involved. Except for Legge and Goadby, who found minute hemorrhages in the peripheral nerves, most workers agree that the usual picture is that of atrophic, degenerative neuritis with subsequent fibrosis.

The broad significance of these pathological changes will be discussed with the probable mechanism which produces lead palsy.

Muscles. Because of its importance in the explanation of lead palsy the pathological condition of the muscles merits careful consideration. Varying degrees of atrophy have been observed by most workers (293) (247) (111) (199) (149) (231), and this condition is generally accepted as a constant accompaniment of lead paralysis (224). It is, however, of considerable significance that these muscles do not merely undergo simple atrophy, but that the changes are

quite similar to those seen in the nerves which supply the affected muscles.

Thus, Legge and Goadby found minute hemorrhages scattered throughout the muscle; Goadby (252) saw fatty degeneration; and nuclear proliferation of the perimysium and loss of cross striations and granular degeneration have been described by various authors (111) (171) (156) (83) (406). Most investigators who have examined the muscles agree with Messing (301) that the lesion is a chronic, degenerative, parenchymatous myositis, with fibrous tissue replacement, and that the changes are not merely secondary atrophy following nerve lesions, but are caused directly by the action of lead.

Blood. A detailed description of changes in the blood during lead poisoning more properly belongs in the chapters dealing with the clinical and physiological aspects of the disease, and will, therefore, be omitted here. But at this point the observation first made by Laennec (244) of the pallor of body tissues after death from lead poisoning and of a diminution of blood in the vessels should be mentioned.

Spleen. As might be expected from the marked hemolytic action of lead, the spleen frequently shows increased pigmentation due to destruction of blood (177) (230). Legge and Goadby state that in their animals the venous sinuses of the spleen were distended with blood. Galvini (151), the only other investigator who reports changes, describes the occurrence of chronic atrophy. Oliver believes that there are no noteworthy changes in this organ due to plumbism.

Bone marrow. Until recently the entire subject of lead anemia has been quite unexplained. Lesions of the bone marrow have been suspected as the underlying cause of this condition.

Stockman and Charteris (445), by administering lead to animals, produced a decrease in the number of fat cells and, after very large doses, observed gelatinous degeneration of the bone marrow. Except for Raimondi (373), no other investigators have obtained such degeneration. In fact, Cadwalader (60) in his one patient, and Ophüls in all his animals, report that a hyperplasia occurred which included the myelocytes, nucleated red cells, and occasionally the megaloblasts. Ophüls also states that there

is increased hematogenous pigmentation of the bone marrow during lead poisoning. Wolff (496) has given a similar interpretation of the changes. He believes that the number of young blood cells increases within the marrow because of a sudden and excessive demand occasioned by peripheral destruction of the blood cells, and he gives no evidence that lead injures the marrow directly. In the light of the clear experimental evidence for destruction of circulating red blood cells during lead poisoning, this hypothesis is of importance.

Bone. The storage of lead in the bones appears to have little or no effect on their structure. Various pathological conditions have been described from time to time but none of them has been proved to have any direct relation to the action of lead on skeletal tissue.

Tanquerel, by gross inspection, could find no abnormal conditions in the joints to explain the frequent development of arthralgia during plumbism. Lewy (264) believed that lead could cause necrosis of the bone, and his belief is confirmed to some extent by the common occurrence of caries of the teeth in lead workers. Osler reported that gouty deposits in the big toe joint might be seen in cases of lead poisoning and thought that the incidence of gout was higher than normal among people who had been exposed to lead.

None of these observations demonstrates that there is any consistent alteration of bone during plumbism, and this seems to support the physiological view that while lead is stored in the bones it is harmless.

Lungs. The supposed prevalence of respiratory diseases among lead workers, particularly of pulmonary tuberculosis, might lead one to expect frequent lesions of the lung in individuals exposed to lead.

Lewy believed that acute asthma, characterized by degeneration, inflammation, and necrosis of the bronchial mucosa, was a common form of trouble in lead poisoning and stated that this disturbance frequently develops into chronic bronchitis, and Greven described endarteritis in the lungs of his rabbits. Glibert (164) has also reported that lesions may appear in the lungs as the result of exposure to lead.

But by far the greater proportion of evidence tends to show, in agreement with Oliver's statements, that no typical pathological changes occur in the lungs as a result of the action of lead.

Generative organs. Like syphilis, lead may cause sterility, abortion, and defective issue. Although exposure of either parent to lead may produce these results, no pathological changes have ever been noted in the ovary. In the male, on the other hand, gradual disappearance of spermatozoa from the gonad, and degeneration and atrophy of the germinal epithelium are frequently seen (486) (32). This picture may occur, however, in many conditions other than lead poisoning. Bell reports that lead causes degeneration of the chorionic epithelium. This, in addition to the action of lead on the uterus itself, must be considered a cause of abortion which is so common among lead workers and among women who take diachylon to cause this condition.

Salivary glands. A few isolated observations have been made on the condition of the salivary glands in lead poisoning.

Apert (11) believes that lead causes a simple hypertrophy of the parotid gland and occasionally inflammatory lesions of the ducts. Rarely periglandular sclerosis has been seen. Allevi (7) thinks that infection of the gland by way of the mouth is a frequent complication of lead poisoning. Lesions in the other salivary glands have never been described.

Glands of internal secretion. Very little has been published about the effect of lead on the glands of internal secretion, probably because these had not attained their present importance at the time when most of the pathological studies were made. The only lesion described in the literature is hypertrophy of the suprarenal cortex (35) (74) (175).

Special senses. The eye is the only sense organ in which lesions have been found in lead poisoning. Such various pathological changes as retinal hemorrhages and acute neuro-retinitis, characterized by swollen, hyperemic discs have frequently been observed. Optic atrophy, however, is the most commonly described lesion (222) (454).

Gibson (157) believes that this condition is due to increased cerebrospinal pressure. Mosny and Harvier (317) report the occurrence of retinal exudation in one case, but they thought that this might be merely the manifestation of a complicating albuminuric retinitis. Some of the temporary amblyopias have been ascribed to spasms of the blood vessels.

Serous membranes. No reports of pathological conditions in the serous membranes of man during lead poisoning have been published in the literature; but Ophüls has produced experimentally a sero-fibrinous polyserositis. This he believes is caused by increased permeability of the blood vessels and fibrous proliferation.

Site of injection of lead. Kumita (242) states that at the site of injection of lead salts calcification occurs in rabbits.

Conclusion. No classification of the various lesions caused by lead can escape criticism, but it seems wise to group the various findings with conservative caution into the four following classes: (*a*) lesions which are found constantly and are caused by lead, (*b*) lesions which frequently occur and are caused by lead, (*c*) lesions frequently seen in subjects with evidences of lead absorption, and (*d*) lesions which have been reported to occur occasionally in subjects with evidences of lead absorption and which may possibly be caused by lead. Under these headings the pathology of lead poisoning may be summarized as follows:

A. Lesions found constantly, due to lead.
    1. Blue line.
    2. Anemia and stippling.
B. Lesions found frequently, due to lead.
    1. Degeneration of anterior horncells.
    2. Peripheral neuritis.
    3. Chronic muscular atrophy and fibrous myositis.
    4. Degeneration of male gonads.
    5. Decrease in fat cells of bone marrow and hyperplasia of leucoblasts and erythroblasts.
    6. Productive meningitis.
    7. Blue patches on mucosa of large and lower part of small intestine.
    8. Optic atrophy.
C. Lesions seen frequently in people with evidences of lead absorption.
    1. Arteriosclerosis.
    2. Ulcers of stomach and intestine.
    3. Contracted small intestines.
    4. Tubular nephritis or chronic interstitial nephritis.
    5. Hemorrhages and exudate of retina and neuro-retinitis.
D. Lesions which have been reported to occur occasionally in people with evidences of lead absorption or lesions possibly due to lead.

1. Vascular hemorrhages.
2. Cardiac hypertrophy, hemorrhages, brown atrophy.
3. Degeneration of unstriped muscle of vessels.
4. Perivenule hemorrhages.
5. Arteritis.
6. Gangrene of extremities.
7. Entero-colitis, acute or chronic.
8. Hypertrophy or sclerosis of sympathetic ganglia.
9. Inflammation and infection of parotid gland.
10. Degeneration, atrophy, cirrhosis of liver.
11. Hematogenous pigmentation of liver and spleen.
12. Hypertrophy of spleen due to erythrophagia or atrophy.
13. Hemorrhagic encephalitis.
14. Neuroglial hyperplasia.
15. Cerebral and spinal cord lymphocytic infiltration.
16. Hemorrhagic myositis, fatty degeneration.
17. Increased pigmentation of bone marrow and gelatinous degeneration.
18. Acute or chronic bronchitis.
19. Gouty deposits and necrosis of bone.
20. Hypertrophy of suprarenal cortex.
21. Hemorrhagic and sero-fibrinous polyserositis.
22. Calcification about local injection sites.

An examination of this pathological picture of lead poisoning indicates quite clearly that definite information about the action of lead can hardly be obtained from such a study. The effect of lead might be considered too subtle to yield to this type of investigation. Certainly, many biological effects are evident which produce no apparent gross or microscopic lesions. The conclusion must be drawn, therefore, that further knowledge of the action of lead on the organism can only be reached by physiological and chemical methods which deal with living rather than post mortem conditions.

Part III. Physiology

XI. Effects of lead on blood cells

Changes in the blood are often the first and most important sign of lead poisoning in man. These effects of lead are stippling and a secondary anemia. Although not an absolute indication of the

severity of the condition, the degree of the anemia and stippling of red corpuscles characterisitic of plumbism usually runs parallel to the state of health. These alterations in the blood doubtless contribute in some measure to the general debility, and therefore possess distinct clinical importance in diagnosis and prognosis. Furthermore, the mechanism of their development throws light on many problems of biological interest.

Stippling of red blood corpuscles. Stippling of the red blood cells, or punctate basophilia, has long been recognized. As early as 1885 Ehrlich (107) described this condition as the presence of basophilic granules in certain red cells. Its association with lead poisoning was, however, first pointed out by Behrend (30) in 1899. The attention of many investigators was immediately focused on the study of stippling, its causation, its relationship to other basophilic conditions such as polychromatophilia and reticulation, and more especially its significance. Because of the completeness of the recently published work of Pappenheim (351), and Naegeli (326), a detailed account of the results obtained and the theories evolved is unnecessary here; but it may be of value to point out some of the salient features of the problems involved and to suggest their significance. This has been done by Key while working in this laboratory, and the following account of stippling is in the main his review of the subject.

Three general questions as to the character of stippling must be considered before discussing its occurrence during lead poisoning: (*a*) are the basophilic granules derived from nuclear or cytoplasmic substance; (*b*) are the changes caused by degeneration or regeneration; (*c*) are these granules related to other basophilic substances, polychromatophilia, and reticulum? After these problems have been solved, the action of lead in the causation of stippling may be studied as a specific example of a general process taking place in the blood.

Origin, formation, and relationship of basophilic granules. The derivation of basophilic granules in the red blood cells was one of the first problems studied.

Ehrlich (107) believed stippling to be of cytoplasmic origin and was supported in his view by Smith (433) and Foa (145). Askanazy (13),

however, in his earlier work thought that he could demonstrate all transitions between polychromatophilia, stippling, karyorrhexis, and karyolysis, and brought forth the theory that the granules of stippling are nuclear fragments. This latter view was generally accepted until Grawitz (176) and his school demonstrated its invalidity by showing quite conclusively that the granules are of cytoplasmic origin. This proof of the correctness of Ehrlich's theory is based on the complete independence of basophilic granules and nuclear substance. Not only have true direct transitions between nuclear fragments and stippling never been observed, but the granules are frequently seen in corpuscles with intact nuclei and in erythroblasts during mitosis. Furthermore, stippling occurs in blood containing no normoblasts (i.e., during lead poisoning), but it is completely absent in the bone marrow where nucleated red cells are most abundant. Grawitz finally pointed out that the granules have none of the characteristics of nuclear substance—they cannot be photographed with ultra-violet light, they are not stained with Ehrlich's tri-acid mixture, and they do take a red stain with Pappenheim's methyl green pyronin.

Since the cytoplasmic origin of these basophilic granules has been definitely established, the question of the nature of their production may be considered.

That stippling is a regenerative process, was the view held by Engle (119) because of the appearance of stippled cells in the embryo. He believed that the presence of basophilic granules in the adult signified a return to the embryonic type of blood formation and was supported in his opinion by Naegeli (326), Koenig (239), and Askanazy (14). Most workers now, however, support the view that stippling is produced by degeneration. The absence of punctate basophilia in the bone marrow (482) (176) (351) (228) (14) (233) (234) as well as the even more significant fact that stippling is associated with pathological processes (176) (249) (30) (377) (398), renders its degenerative character quite probable.

To understand clearly the nature of stippling, its relation to the various other basophilic substances of the cytoplasm must be studied. Many evidences of the close alliance to the polychromatophilic substance may be found, and these have been summarized by Key as follows.

The same fixatives are effective for both, and the same differential stains. Both are soluble in alkalies, and insoluble in water and various acids; both

contain potassium, but neither contains iron, using McCallum's (287) method for the detection of these elements; and both give a slightly positive Millon's test. Finally, in a single preparation all transitions between polychromatophilic cells, stippled polychromatic and stippled orthochromatic cells may be demonstrated. Reticulum also has the same staining and chemical characteristics. Its relation to stippling may be demonstrated by drying smears incompletely and staining them in an isotonic medium. In such preparations all stages between stippled fragments and typical reticulum may be observed. By incomplete fixation with osmium tetroxide vapor all transitions from fragmentation to diffuse polychromatophilia (233) may be demonstrated. Consequently the close relationship between stippling, reticulum and the polychromatophilic substance is apparent.

The general significance of stippling. All the facts thus far mentioned point strongly toward the view that stippling is a granular degeneration or coagulation of basophilic substance. To throw more light on this question, Key (234) performed some interesting and convincing experiments which have an important bearing on the significance of stippling.

He administered lead to two groups of rabbits—one normal and the other made anemic by previous intermittent bleeding. In normal animals the marked reduction in the number of red blood cells which occurred promptly after giving lead was followed by a striking increase in the number of circulating young cells. This indicates that the anemia was caused primarily not by suppression of bone marrow activity but by destruction of existing blood cells. In the anemic group of animals the diminution in the red cell count after exposure to lead was much less than in those which were not bled. In fact, in some of the anemic rabbits there was a transitory rise in the cell count above that obtained before lead was given. This Key explained as due to a probable stimulation of bone marrow. The nature of this stimulation is not definitely known but may depend on the fact that the frequent bleedings make constant demands upon the bone marrow and render it hyperactive. In such a condition a transitory and slight destruction of blood by lead might call forth excess blood formation very rapidly. Stippling appeared in the blood of both groups of animals within twenty-four hours after administration of lead and persisted for ten days to three weeks or more. Simultaneous counts of the red, reticulated, and stippled cells demonstrated the very significant fact that the number of stippled

cells varies directly with the number of young cells and is much greater in anemic than in normal animals.

By these experiments on rabbits, Key practically proved that stippled cells are young red corpuscles which are exposed to lead and that the granules are probably aggregations of part of the basophilic substance into small discrete masses. This reaction is probably degenerative in its nature. That stippling is not an artefact produced by staining, Key demonstrated by examining fresh blood under the microscope. In this he found cells containing slightly refractile clear masses exhibiting Brownian movement. The number of these corresponded roughly to the number of stippled cells present in stained preparations of the same blood. He also thought that by a modification of the McJunkin (290) method of staining he could demonstrate that stippling is not precipitated lead within the cell because many stippled cells contained no black granules of lead sulphide and most of the cells containing these granules were not stippled. Moreover, both stippling and precipitated lead may occasionally be seen within a single cell.

This method consists of fixing smears of blood in a mixture of alcohol and ether for twenty-four hours and exposing them for thirty minutes to a 10 per cent NH_4OH solution saturated with H_2S. They are then counterstained with eosin or, better still, pyronin. It must be emphasized, however, that the amount of lead in blood is so very small that the actual interpretation of black particles as lead sulphide must be made with very great caution, if at all.

This rather detailed discussion of the derivation of the granules in stippled red blood cells is pertinent here because it emphasizes the pathological nature of stippling and its development as the result of a toxic action on the young cells. Since stippled cells are never found in the bone marrow, even when they are very numerous in circulating blood, this toxic action apparently occurs peripherally.

Stippling as a clinical phenomenon. Before discussing the mechanism of the production of stippling by lead, it is essential to point out that the presence of punctate basophilia in the blood is not a specific sign of lead poisoning.

It occurs in pneumonia in infants (58), in pernicious anemia, leucemia, pseudoleucemia, in the anemias due to cachexia of neoplasms, and in the anemias caused by hemorrhage when the hemoglobin is retained in the organism (176). Stippling also may be seen in the blood of embryos of mice (118), rabbits, guinea pigs, sheep and swine (326), but is rarely found in human embryos (239). Hamilton (195) reports that it has been observed in cases of aniline poisoning. Thus, stippling is not caused by the action of lead alone, but may be produced by other toxins and perhaps during normal embryological conditions. This suggests that stippling represents a general pathological reaction of the cell rather than some specific phenomenon.

But the occurrence of stippling in the red cells in these various other conditions is relatively rare and slight as compared with the frequency and intensity of its appearance during plumbism. For this reason the demonstration of stippled red cells in the blood has come to be considered an almost definite evidence of absorption of lead. Many clinicians even go further than this and consider stippling the most important sign for the diagnosis of lead poisoning (See section XVII).

Methods of staining stippled cells. The choice of methods for staining red blood cells to demonstrate stippling is of importance, because unsuitable or poorly applied stains may fail entirely to show basophilic granulation.

Ordinary differential stains such as Wright's, Jenner's, and Hasting's may not bring out the basophilic granules even when there are many stippled cells. Over-staining with these so that the effect of methylene blue is pronounced usually demonstrates the granules. Therefore, to obtain good pictures of punctate basophilia with these stains, the smears must have a distinctly blue tinge so that the neutrophilic granules stand out clearly. Hayhurst (204) prefers Skelton's or Harlow's method of staining a dried blood smear with eosin (1 per cent absolute methyl alcohol solution) for one minute, then with methylene blue (1 per cent absolute methyl alcohol solution) for one minute, and washing free with water. Schwarz (407) recommends the examination of a thick blood smear for a rapid diagnosis, and Seiffert (415) suggests staining the unfixed smear with Loeffler's methylene blue. This latter method is very objectionable, according to Engel (118), because unfixed smears stained with methylene

blue show many granules in normal cells. Seiffert (416), however, believes that real stippling can easily be differentiated from these false granules. These "pseudo" granules are pale, rather irregular, and are usually seen in a network of reticulum, while true granular basophilia is very dark and may be intensified in the fixed preparation. To be significant in the diagnosis of lead poisoning Seiffert holds, true stippled erythrocytes must be found in every five or six fields. Schreiber (405) uses fresh preparations of blood which are stained with a complex "combined brilliant cresyl blue Giemsa dye," and Sellers (417) mixes a drop of fresh blood with a drop of 1 in 500 methylene blue on a slide. In our work the best results in demonstrating the granules were obtained with Unna's alkaline methylene blue solution, which has been employed by many investigators in this field. It consists of one part methylene blue and one part potassium carbonate per hundred parts of water, and is used as follows. Dried thin blood smears, which have been fixed with methyl alcohol and dried, are flooded with a 1:15 dilution of the stain and allowed to stand for fifteen minutes. The stain is then washed off with water until only a faint bluish green tinge remains. When prepared in this manner the red corpuscles appear light greenish blue, the nucleus of leucocytes and the basophilic granules dark blue. The stippled cells usually are somewhat polychromatic.

The general biological problems related to stippling. As this survey shows, some of the fundamental problems of stippling are still unsolved, and several questions of both biological and practical significance merit further investigation. For instance, basophilic granules are considered to be derived by some degenerative process from the basophilic substance of young corpuscles which normally gives the cells their polychromatophilic appearance. But lead has never been known to produce stippling *in vitro* (393) (439) (234). Another suggestive problem is the apparent restriction of stippling to certain species. In man, rabbits, guinea pigs, and rats, lead poisoning is characterized by the appearance of basophilic granules in the red blood corpuscles, but in the blood of cats, chickens, pigeons or amphibia stippling has never been produced (234) (303) (196) (328) in spite of the fact that the corpuscles of amphibia and birds are known to contain basophilic substance. White and Pepper (491) report an atypical stippling in dogs poisoned with lead. The granules, however, were brought out after heating the smears and were clumped and large, quite unlike the usual picture. Also the fact that they

describe stippling in bone marrow cells, which has not been obtained by other workers, makes their finding of stippling in dog's blood very questionable. In our work innumerable examinations of rabbit's (234), rat's and man's blood have demonstrated without question the occurrence of punctate basophilia in these species but distinct stippling has never been seen in the blood of cats even when symptoms of plumbism are very marked. It is true that occasionally a suggestive mottling appeared but certainly no definite stippling. The broader significance of this will be emphasized later in a discussion of the relation of stippling to other blood changes caused by lead, but it is sufficient here to indicate that failure to produce stippling *in vitro* suggests that this is not a simple phenomenon. Furthermore, the occurrence of stippling in some animals and its absence in others implies a biological and perhaps chemical variation between red blood cells of different species.

Anemia. The other important change in the blood caused by lead—the anemia—has long been recognized and repeatedly demonstrated. As early as 1831 Laennec (244) described pallor of tissues and thinness of blood in cases of plumbism at autopsy. In 1840 Andral and Gavarret (8) first actually counted the number of red blood cells during lead poisoning and found the count much lower than normal. Now an erythrocyte count is a routine clinical procedure in lead intoxication. In fact, in some experimental work anemia is considered an index of the degree of lead poisoning in the animals. A study of lead anemia is important not only because of its clinical significance, but also because a clear understanding of the action of lead on the red blood corpuscle, as an isolated cell, throws considerable light on the mechanism of its reaction with other body cells.

This anemia has been attributed both to peripheral destruction of blood and to lesions in the bone marrow which result in insufficient production of blood cells.

The latter explanation was supported by Raimondi (373) and by Stockman and Charteris (445) who described a preliminary hyperplasia of bone marrow followed by definite gelatinous degeneration. Wolff (496) also believed that the bone marrow is affected but not in the early stages of plumbism; while Meillère (293), Schnitter (402), and Sellers (417) all stated that lead definitely injures both blood corpuscles and bone marrow. These

are the only workers who thought that lead may interfere with blood formation. But, although bone marrow may possibly degenerate in advanced lead poisoning, the preponderance of evidence indicates clearly that the anemia is probably caused by destruction of cells in the circulating blood—a fact first suggested independently by Heubel (213) and Bouchard (49), and later by Rauch (377) and others. The evidence for this is quite conclusive. Early in lead intoxication there is hyperplasia of bone marrow, and the large numbers of nucleated and reticulated red cells in circulation indicate that regeneration is taking place. Key (234) has shown that in rabbits within twenty-four hours after the ingestion of 1 gram of lead the number of circulating red cells decreases more than 20 per cent, but that many nucleated and reticulated erythrocytes appear. That hematopoiesis in lead poisoning is not impaired can also be demonstrated by a reticulated count as a measure of young cell formation. This was 6 per cent and 16 per cent in two of our lead colic patients who had red counts of approximately 3,500,000 cells. These figures indicate clearly that the bone marrow was actively combating the anemia.

Excretion of the decomposition products of the red blood cells has also been studied.

As long ago as 1871 Heubel (213) obtained evidence of the direct action of lead on blood by finding increased pigment in serum, bile and urine. Meillère (293) observed a definite increase in the urinary excretion of iron which is largely derived from red corpuscles. That the presence of hematoporphyrin, a product of blood destruction, in urine is an important factor in diagnosing plumbism is a fact more commonly known in Europe than in this country. Brady (52), in this laboratory, demonstrated very marked hematuria and an increase in the excretion of bile pigments from the common duct of rabbits poisoned with lead acetate. This, however, was given in rather massive doses, 1.8 gram by stomach tube in some experiments and 125 mgm. intravenously in others, and represents a very acute poisoning. In human cases, Jones (229), using the regular Lyons' method, has obtained similar evidence of marked destruction of circulating blood. He found that the bile obtained by duodenal tube as well as the blood plasma from several patients suffering from lead poisoning contained much higher concentrations of bile pigment than that from normal subjects. All these results point to the probability, therefore, that lead acts on the peripheral blood and ordinarily has no primary inhibitory effect on hematopoiesis.

Experimental Observations. Since lead seems to affect directly

the corpuscles in the circulating blood, it is of prime importance to understand the exact chemical and physiological nature of the reaction. For the last three years therefore, we have studied this problem (15) (16) (17), and many *in vitro* and *in vivo* experiments were performed which afforded an explanation of the anemia of lead poisoning.

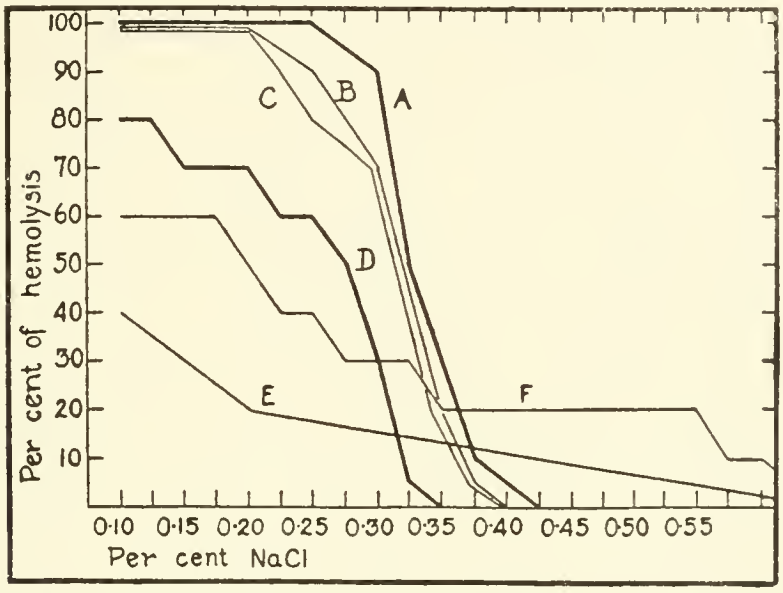


FIG. 24. SODIUM CHLORIDE HEMOLYSIS. THE EFFECT OF VARYING THE QUANTITY OF LEAD

The curves show the effect on hemolysis of previous exposure for one hour of washed red blood cells to:

B, 1 cc. of red blood cells exposed to 0.002 mgm. of Pb as chloride.
C, 1 cc. of red blood cells exposed to 0.004 mgm. of Pb as chloride.
D, 1 cc. of red blood cells exposed to 0.01 mgm. of Pb as chloride.
E, 1 cc. of red blood cells exposed to 0.03 mgm. of Pb as chloride.
F, 1 cc. of red blood cells exposed to 0.08 mgm. of Pb as chloride.
A shows the action of the control normal red blood cells in Ringer solution. This chart indicates the per cent of hemolysis in each concentration of salt solution.

Hemolysis in hypotonic saline solution. One of the most striking phenomena observed in this work is the increased resistance of red blood cells to hypotonic saline solutions after exposure to lead.

As long ago as 1873 Malassez (275) noticed not only that anemia was a symptom of lead poisoning but that the blood cells were larger and thicker than normal. Agasse-Lafont and Heim (2) have found slightly increased globular resistance in cases of plumbism, and von Liebermann (266), Orbán (343), and Hayhurst (205) have confirmed this observation. In an

elaborate investigation Fici (135) has recently demonstrated the phenomenon *in vitro*. He also found that lead acetate not only increases the resistance of some cells to hypotonic saline solution but makes other corpuscles more fragile and suggested that these phenomena are due to the direct action of lead on the cell. His work is very interesting in the light of our experiments.

In the experiments in this laboratory, washed human corpuscles from defibrinated blood were used and portions of these were exposed to lead as lead chloride in Ringer solution, the rest being treated with regular Ringer solution as controls. The pH of all solutions was kept at 6.5 and all manipulations were so standardized that they did not vary from day to day. In every experiment the control tests allowed direct comparisons—a method which gives more accurate results than would comparisons with an average normal. The so-called "fragility" or hemolysis tests to hypotonic saline were performed on the "leaded" and control cells. Using this method, we found that exposure to a concentration of only two parts of lead, by weight, per million (1 cc. of corpuscles per 0.001 mgm. lead) definitely increased the resistance of cells, while a solution of one part per hundred thousand (1 cc. of corpuscles per 0.01 mgm. lead) produced so marked an effect that many of the corpuscles did not hemolyze completely in 0.1 per cent saline. Normal cells, on the other hand, usually are completely hemolyzed by 0.25 per cent salt solution. This result is illustrated by figure 24. It is striking that in the three tubes corresponding to those in which normal cells were first destroyed, there was no hemolysis of the least resistant "leaded" corpuscles. If greater concentrations of lead were used, two phenomena at once became evident. The resistance to hemolysis increased but some cells were so injured that they hemolyzed upon standing even in normal Ringer solution, although the controls did not (fig. 24). The action of lead is therefore double: (*a*) the cells exposed to lead withstand greater change in osmotic tension, but (*b*) they break up more rapidly than normal cells.

If the results of a series of these experiments dealing with hemolysis of cells in hypotonic saline solution are plotted (fig. 25) so that each point represents the increase in per cent of hemolyzed cells, it appears clear that the cells are unevenly "leaded" or that two antagonistic

reactions are involved, one of which permits hemolysis in salt solution at concentrations above 0.225 per cent, while the other prevents hemolysis until the concentration falls to 0.1 per cent. The invariable movement toward the left of curves representing hemolysis of "leaded" corpuscles suggests that the normal cells which hemolyze most easily are those most susceptible to lead and are rendered more

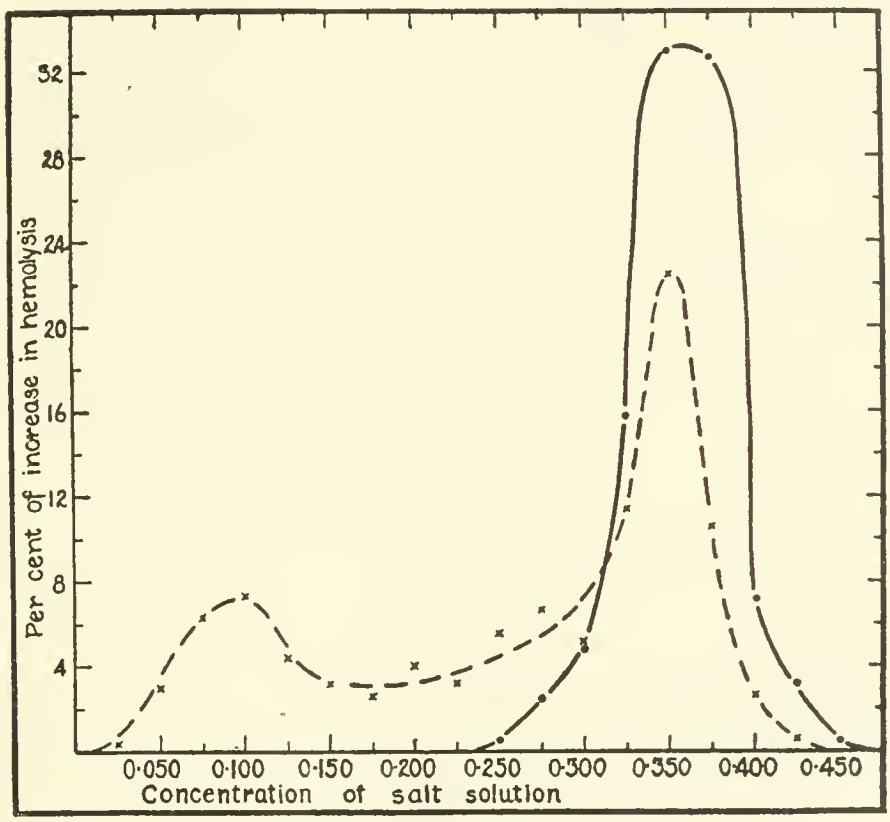


FIG. 25. THE DISTRIBUTION OF HEMOLYSIS. THE AVERAGE OF TEN EXPERIMENTS WITH CELLS EXPOSED TO 0.01 MGM. OF LEAD AS LEAD CHLORIDE PER CUBIC CENTIMETER OF WASHED RED BLOOD CELLS FOR ONE HOUR

Each point represents the per cent increase of hemolysis over that in the next tube of greater saline concentration.

●———● normal cells. x - - - - - - x "leaded" cells.

resistant by its action. Which cells these may be—reticulated or mature, young or old—has not yet been definitely demonstrated.

Attempts to reverse the effect of lead in changing the hemolysis of cells in hypotonic saline solution by "de-leading" them have proved unsuccessful and therefore suggest that the lead reaction is irreversible. Moreover, our experiments show that the salts of other metals (cerium, aluminium, calcium, arsenic, cadmium, copper,

mercury) or substances like formalin and tannic acid have no comparable effect; whereas the other soluble lead salts, the nitrate and acetate, have the same effect as lead chloride. It is, therefore, probable that the action of lead on the resistance of red blood cells to hypotonic salt solution is an isolated phenomenon, at least in its intensity under these conditions. Furthermore, this modification of the fragility of "leaded" cells in hypotonic saline solution affords a delicate method for the determination of the biological effects and chemical reactions of lead. This reaction may be obtained in whole blood but more lead must be added than is necessary with washed red cells.

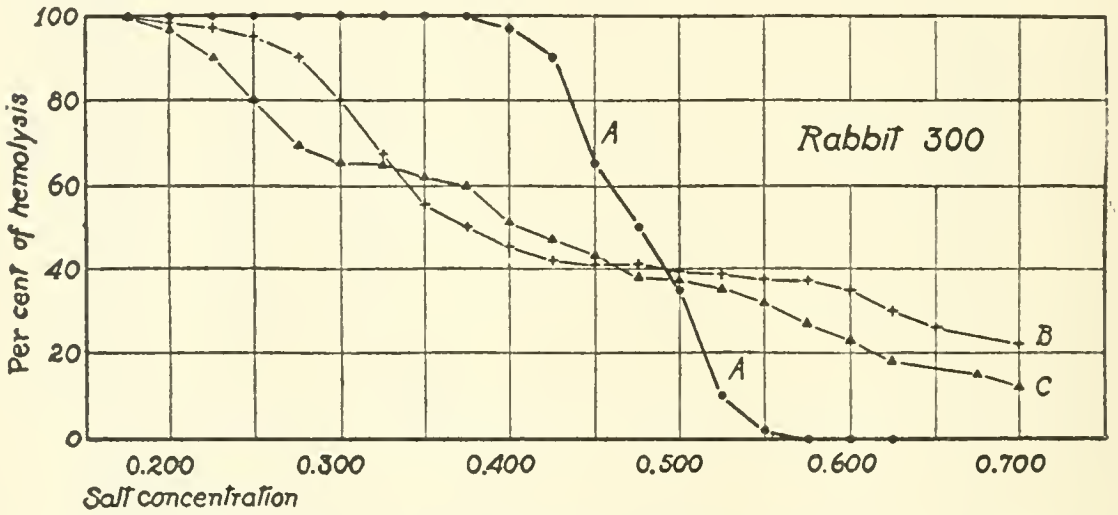


FIG. 26. SODIUM CHLORIDE HEMOLYSIS. THE EFFECT OF ACUTE LEAD POISONING ON RED BLOOD CELLS IN VIVO

The curves represent the action of red blood cells washed in Ringer solution.
A, before lead was given.
B, nineteen hours after 1 gram of lead acetate was given by mouth.
C, forty-three hours after 1 gram of lead acetate was given by mouth.

If this reaction between lead and red corpuscles has any great significance its occurrence in life must of course be demonstrable. That it is, has already been shown in man by von Liebermann (266), Orbán (343), and Hayhurst (205) who observed that corpuscular resistance increases during acute lead poisoning. Further investigations of the resistance of corpuscles have been carried out on rabbits. Figure 26 shows the results obtained, and demonstrates that *in vivo* the corpuscles are affected just as *in vitro*. Apparently many "leaded" cells resist the strain of hypotonic saline better than the control

corpuscles but some hemolyze quickly even in normal saline solution. This justifies the assumption that the phenomena studied here *in vitro* are similar to those which may occur in life and are most probably related to development of the anemia of lead poisoning.

Further evidence of connection between the modification of the hypotonic saline curve and the changes caused by lead *in vivo* is

TABLE 33

The parallelism between blood manifestations in various animal species

The effect of lead *in vivo* on the appearance of anemia and stippling in blood, and *in vitro*, on the hemolysis in hypotonic salt solution

SPECIES	DISTORTION OF HYPOTONIC SALINE CURVE TO LEFT FOLLOWING EXPOSURE TO LEAD*	APPEARANCE OF ANEMIA IN "LEADED" ANIMALS†	APPEARANCE OF STIPPLING IN "LEADED" ANIMALS†
Man	++	+	+
Rabbit	++	+	+
Rat	+++	+	+
Guinea pig	+	+‡	+‡
Dog	Negative	?	?§
Cat	Negative	Negative until just before death	No clear cut stippling. Rare mottling of cells
Horse	Negative	?	?
Chicken	Negative	?	Negative.‖

* All exposed to same concentration, *i.e.*, 0.01 mg. of Pb per cubic centimeter of washed red blood cells. Plus means that the curve of "leaded" cells indicated distinctly increased resistance to hemolysis in dilute saline solutions.

† Concentrations of Pb per kilogram of animal not constant.

‡ Data from Sabrazès, Bourret, and Léger (393), and from Beresina and Engling (32a).

§ Stated by White and Pepper (491) to be positive, though not typical. This observation has not been repeated.

‖ Data from Key (234) and also from Meyer and Speroni (303).

indicated by the fact that the action of lead on hemolysis in hypotonic solution seems to run parallel to the appearance of stippling and anemia in different species of animals. Table 33 shows that these phenomena have not been observed in all species but that in those studied all are either present or entirely lacking. Thus, in man, rabbits, guinea pigs, and rats, stippling and anemia are easily produced and there is the characteristic change in the resistance to hemolysis in hypotonic salt solution after "leading." The red cells of horses,

dogs, and cats, on the other hand, show no changes in resistance as the result of adding lead *in vitro*. The effect of lead on the blood of horses had not been studied *in vivo* but the cat shows no signs of anemia and no definite stippling has ever been observed in its blood. In the case of the dog, the occurrence of anemia has never been reported and the presence of atypical punctate basophilia in this animal rests on the work of White and Pepper (491). Tests to determine whether lead does react with cat's blood without apparent physiological effect were carried out by mixing a known quantity of lead with a given volume of cat's washed red blood cells. The mixture was then centrifuged and the supernatant fluid treated with fresh human corpuscles. Hypotonic saline tests were performed and were entirely negative for lead effect. These experiments, therefore, show that the lead reacts with the corpuscles even though their resistance to hypotonic salt solution is not affected.

It may be well to consider these results and their general physiological significance more fully. Three apparently distinct phenomena are involved—stippling, anemia, and changes in resistance to hypotonic saline solutions—yet the occurrence of all in certain animals and the complete absence in others points to some interrelationship. Probably all three depend on one underlying cause. Certainly this correlation between the hypotonic saline test and the other blood phenomena which occur *in vivo* points to the fact that this modification in the hemolysis of "leaded" cells in hypotonic saline represents a real physiological reaction and not an accidental *in vitro* change. The variations in the properties of cells of different species found in these experiments suggest many possibilities and offer an attractive field for further investigation.

Chemistry. The action of lead on the red blood cell is probably chemical in its nature, and, as has been pointed out, the hypotonic saline test affords an excellent means of studying such a reaction. Lead can no longer change the resistance of red blood cells to hypotonic saline solution if mixed with a small amount of blood serum before being added to the corpuscles. In other words, some substance in the serum so reacts with lead as to prevent combination with red blood cells subsequently added. Using this finding as a basis, each of the known constituents of red blood cells (sodium

bicarbonate, inorganic phosphate, lecithin, cholesterol, euglobulin, pseudoglobulin, hemoglobin, and albumin) in pure form and in an amount equivalent to that in a given quantity of blood was mixed with lead. After allowing time for reaction between the lead and the blood constituent, red cells were added to the mixture and the usual hypotonic saline test was carried out. A normal curve indicated that the blood constituent had obviously so combined with lead during the preliminary treatment as to prevent the action of lead on the blood cells. On the other hand, if the curve was that characteristic of "leaded" cells there had been no interaction between the substance tested and the lead. In all experiments, the solution of blood constituents simulated as far as possible the conditions in the blood, especially with respect to hydrogen ion concentration and osmotic pressure. The exact constituents of the blood cell are not definitely known, certainly not their quantitative distribution. In fact, the presence in the corpuscle of some of the substances mentioned has been questioned. But in all this work the generally held opinion of the chemical nature of the red blood cell has been accepted. Results of these tests show definitely that inorganic phosphate, in the concentration normally found in serum or corpuscles, neutralizes the same quantity of lead as does whole serum. This can, therefore, completely account for the action of lead on red blood cells. Sodium bicarbonate also neutralizes lead, but ten times as much as occurs in an equivalent neutralizing quantity of serum is required. None of the other constituents tested under these conditions combined with lead. The failure of lecithin to unite with lead, as well as negative results with sodium glycerophosphate, indicates that such organic or bound phosphate plays no direct part in the action of lead.

There is still further evidence that lead unites with the inorganic phosphate of the blood. Serum with increased inorganic phosphate content, obtained from nephritic patients, neutralizes the effect of lead to a high degree. Moreover, when inorganic phosphate diffuses from red blood cells into the surrounding Ringer solution lead no longer acts upon the corpuscles because of its neutralization by the diffusate. If these cells are washed free from the diffusate, however, lead can again react with them. These phenomena indicate that the action of lead upon red blood corpuscles can be entirely explained

by the union of lead with the inorganic phosphate of the cell. It is important to point out here that when a lead salt unites with phosphate the very insoluble $Pb_3(PO_4)_2$ is formed with the liberation of free acid.

$$3PbCl_2 + 2Na_2HPO_4 \rightarrow Pb_3(PO_4)_2 + 4NaCl + 2HCl$$

Fragility. With this chemical picture in mind it is of interest to consider some of the physiological changes which occur when lead acts on corpuscles. The physiological action of lead on the red blood cell is very striking. If red corpuscles, treated with a lead salt, are permitted to stand in Ringer solution, an abnormally large number of these "leaded" cells hemolyzes within a few hours. This demonstrates that lead shortens the life of the cell. The increased fragility after exposure to lead, when cells are rotated or treated with carbon dioxide, is even more marked and is evidence of the fact that slight trauma can destroy "leaded" cells very easily. Rous and Robertson (391) and recently Broun (57) have attributed destruction of blood cells to mechanical trauma, and these experiments illustrate that cells injured by lead are very much more fragile than normal, and therefore far more susceptible to the trauma of circulation. This is probably a very important factor in the increased destruction of blood in plumbism.

Specific hemolysis. Anti-human serum, obtained from a rabbit immunized against human blood, caused much more rapid and complete hemolysis of "leaded" cells than of control cells. The significance of this is not yet clear, but may have some bearing on the explanation of mechanisms of various reactions which occur in hemolysis.

Agglutination. One of the important practical problems connected with blood is the agglutination of corpuscles by heterologous sera. Experiments performed on human blood with Group 2 or Group 3 corpuscles and Group 4 serum consistently showed that after exposure to lead agglutination is slow and slight, if it occurs at all, whereas in the control tubes exposed merely to Ringer solution strong agglutination follows the addition of serum (fig. 27).

Stickiness of red blood corpuscles. The discovery that lead inhibits agglutination of red blood cells in heterologous serum naturally raises

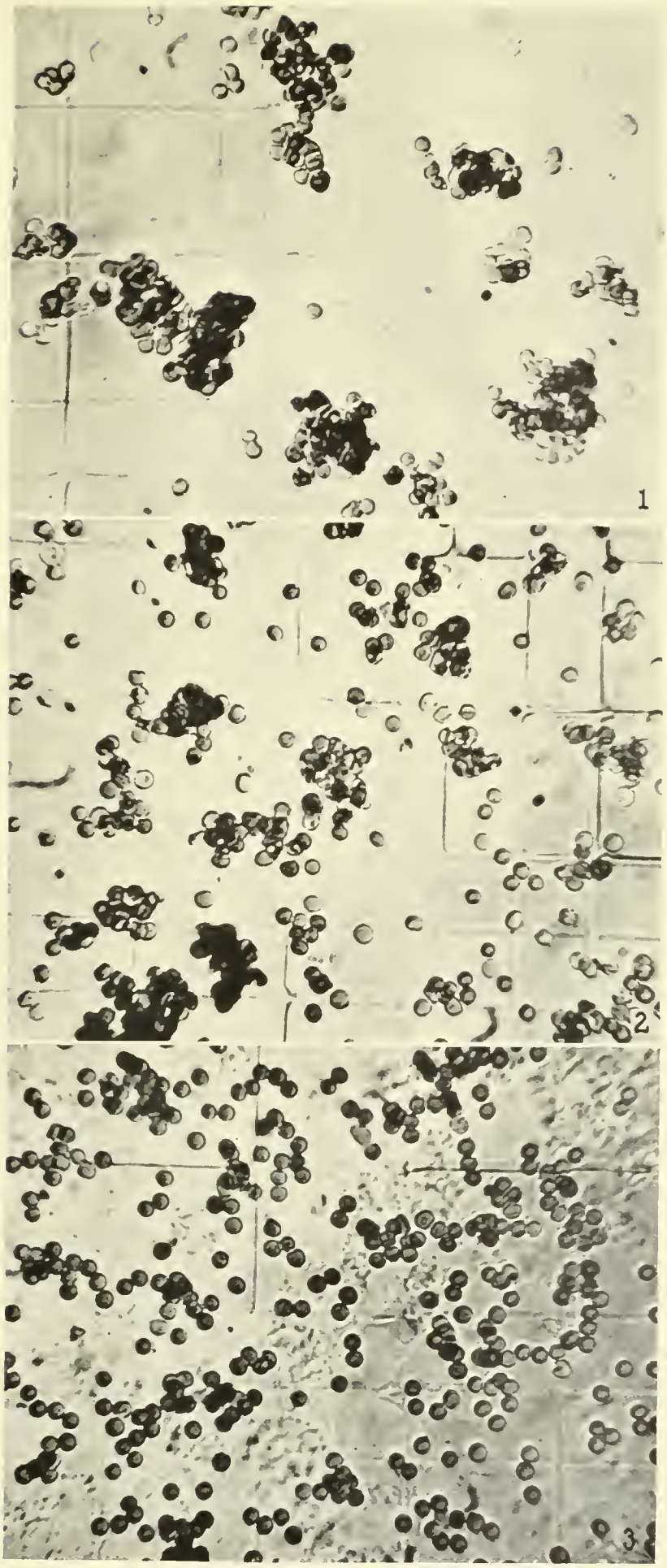


FIG. 27. EFFECT OF LEAD ON THE AGGLUTINATION OF GROUP II CELLS BY GROUP IV SERUM

1. Normal cells exposed only to Ringer solution.
2. Cells exposed for 1 hour to 0.015 mgm. of Pb per cubic centimeter of cells.
3. Cells exposed for 1 hour to 0.05 mgm. of Pb per cubic centimeter of cells.

the question of whether it affects the natural stickiness of the cell surface or whether the addition of agglutinating serum causes the change. This problem was studied by means of Fenn's (133) method which consists in placing a suspension of cells in a glass chamber which is rotated 180° after a given interval. The number of cells which remains on the ceiling of the chamber, as well as the number which falls off, is then determined under the microscope. It was found that normal control cells remained on the ceiling of the chamber after rotation, whereas a large proportion of "leaded" cells fell. From this the conclusion may be drawn that after exposure to lead the surface of red blood corpuscles is less sticky than normal. There are three possible explanations for this. In the first place the lead may have added weight to the cell. This is hardly probable because of the very small amounts used. However, the effect of the lead is actually to increase the specific gravity of the corpuscle due to a marked shrinking (see page 148). But this increase in weight is probably not the important factor in decreasing the stickiness of "leaded" cells because the same phenomenon is illustrated in the changes with iso-agglutinins where variations in specific gravity are of no significance. Furthermore, this difference in stickiness between "leaded" and normal cells does not occur unless serum is added to the media. If the variation was merely due to changes in weight, it should be seen in any medium. The other possibilities which must be considered as causing this loss in stickiness are that the charge of the cell surface may be changed (see page 149), or that because of alteration in its chemical composition, the surface may have changed its physical characteristics (see page 151).

In the case of polymorphonuclear leucocytes, obtained from the peritoneal cavity of rats after aleuronat injections, no definite difference between the stickiness of "leaded" cells and that of normal controls could be observed. It therefore seems probable that lead affects the surface of white and red cells differently.

Phagocytosis. This finding is especially interesting in view of the work of Fine (137).

He studied the phagocytizing ability of rat's white blood cells for particles of metallic lead, basic lead carbonate, lead chromate, and lead sulphide,

using the "film method" of Fenn (132). The number of particles ingested by the leucocytes is determined and thus the rate of phagocytosis. Fine found that metallic lead is less readily phagocytozed than the lead salts and suggests the possibility of inhaled metallic lead acting as a foreign body in the lung and exciting fibrosis.

These results brought up the question of the possible effect of soluble lead on the phagocytozing power of the leucocytes. To study this the leucocytes were exposed to varying concentrations of lead chloride and the rate of phagocytosis for basic lead carbonate particles was determined. Although only two experiments were carried out in which the cells were subjected to the lead chloride for more than twenty minutes, these show very definitely that lead chloride in as low a concentration as 0.02 mgm. of lead per cubic centimeter of suspension is able to retard the ingestion of particles very markedly, which demonstrates rather definitely that soluble lead injures the leucocyte.

The problem that these results bring up suggests many implications which cannot be discussed here. Of particular interest, however, is the failure of lead to affect the surface of the white blood cell, indicated by the stickiness experiments, and its inhibiting action on their phagocytozing power. Of course it must be remembered that the results of the stickiness experiments on white cells is only negative evidence and may simply mean that a change in the surface could not be detected by this method. The conclusion seems evident, therefore, that either variation in stickiness is not an absolute indication of surface changes, or that lead can interfere with certain functions of the white blood cell and leave its surface unimpaired.

Permeability of the cell surface. The salt hemolysis test is based upon the permeability of the cell to water, and the elasticity of its surface. When osmotic tensions of surrounding fluids vary, the cell swells or shrinks because of changes in water content, and only its elasticity determines the degree of change of osmotic pressure which it can withstand without hemolysis. Since the cell responds thus, by changing its volume, the specific gravity of normal and "leaded" cells must be an index of their permeability to water and therefore of changes in elasticity. Using the benzyl benzoate-oil method

(384)[2] it was found that "leaded" red blood cells always have a distinctly higher specific gravity than normal cells. The specific gravity of normal red blood cells varies between 1.088 and 1.096 (average 1.091), that of "leaded" cells between 1.104 and 1.113 (average 1.108), a difference sufficiently great to be well outside

TABLE 34

The specific gravity of red blood cells after exposure to lead for varying lengths of time

TIME	SPECIFIC GRAVITY
15 minutes	1.089
30 minutes	1.098
1 hour	1.115
2 hours	1.113
3 hours	1.112

TABLE 35

Effect of osmotic changes on specific gravity

CONCENTRATION OF RINGER SOLUTION		SPECIFIC GRAVITY OF		CHANGE OF SPECIFIC GRAVITY FROM CELLS IN NORMAL RINGER SOLUTION		TOTAL CHANGE OF SPECIFIC GRAVITY	
Before and during "leading"	After "leading"	Normal cells	"Leaded" cells	Normal cells	"Leaded" cells	Normal cells	"Leaded" cells
0.9	0.9	1.089	1.111				
0.9	0.5	1.065	1.103	−24	−8		
0.9	1.35	1.109	1.115	+20	+4		
0.5	0.9	1.092	1.113	+3	+2		
1.35	0.9	1.090	1.114	+1	+3		
0.9	0.9	1.089	1.106				
0.5	0.5	1.069	1.097	−20	−9		
1.35	1.35	1.104	1.110	+15	+4		
0.5	1.35	1.107	1.115	+18	+9	+38	+18
1.35	0.5	1.073	1.095	−16	−11	−31	−15

the limits of experimental error. This increase in specific gravity, as would be expected, develops slowly and becomes maximal only

[2] This method depends upon the suspension of a small drop of corpuscular cream, centrifuged free from serum, in mixtures of benzyl benzoate and cotton-seed oil. By varying the proportions of these two fluids, media of known varying specific gravity are obtained. The one in which the red blood cells neither rises nor sinks, but remains suspended, is equal to the specific gravity of the corpuscles.

after an hour (table 34). Since this change may be observed after the addition of a quantity of lead as small as 0.01 mgm. per billion cells, it cannot be explained by the mere increment of the heavy metal. It must be due to an actual increase in the density of the cell caused by a decrease in its volume after exposure to lead although the osmotic tension of the surrounding fluid is not altered.

When the osmotic tension of the fluid is varied and the specific gravity of the cells determined, very interesting differences between normal and "leaded" cells appear. Figure 28 and table 35 show the result of placing normal and "leaded" cells in concentrations of 0.5, 0.9, and 1.3 per cent Ringer solution. "Leaded" cells proved to be heavier in every case. Their range of swelling and shrinking

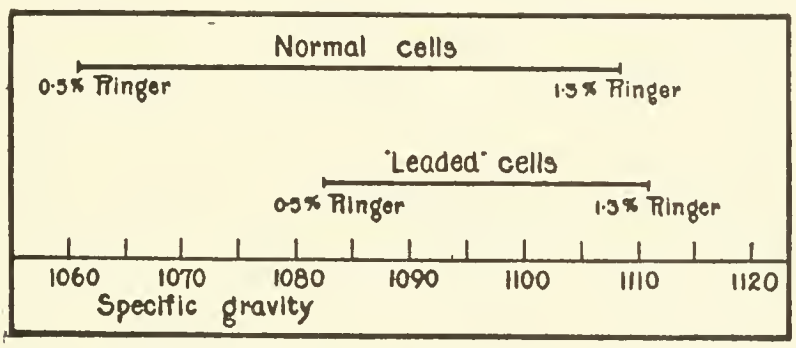


FIG. 28. EXPERIMENT DEMONSTRATING TOTAL CHANGES OF SPECIFIC GRAVITY OF CELLS IN VARYING CONCENTRATIONS OF RINGER SOLUTION

is far less than normal, but they are not impervious to water for some variations in weight may be observed and the usual specific gravity is regained in 0.9 per cent Ringer solution even after exposure to solutions of other concentrations. The difference observed, therefore, is apparently due to the fact that the exchange of substances through the cellular membrane is restricted—decreased permeability to water. The most reasonable explanation for this phenomenon is that the cell surface probably shrinks after exposure to lead and becomes less elastic or that the charge of the cell surface is so changed as to repel a definite quantity of water.

A second method used to study the difference in swelling capacity between normal and "leaded" cells was to determine the volume of a unit number of corpuscles of each kind. This was based on the assumption that the number of cells in a unit volume would vary with

the size of the cell. Normal corpuscles and those exposed to lead were changed from a 0.9 to a 0.6 per cent medium. From the red cell counts and hematocrit determinations of the control and "leaded" cells in both 0.9 and 0.6 per cent Ringer solutions, the number of corpuscles packed into a unit volume in each case could be obtained and thus the change in cell volume. The results from such experiments cannot be considered more than a check on the more exact specific gravity experiments because of the increasing error involved in cell counts as blood is diluted. However, in two cases in which the theoretical values closely approximated those obtained it was found that normal cells could swell 19 per cent in one experiment and 28.4 per cent in the other when changed from a 0.6 to a 0.9 per cent medium. The "leaded" cells in the first case could swell only 0.06 per cent and in the other 6.6 per cent. Thus, the inability of "leaded" cells to swell, except within narrow limits, is apparent.

The appearance of the cell. The specific gravity experiment suggests that microscopic examination of the corpuscles might demonstrate a change in size after exposure to lead. That this is true was shown by measuring large numbers of cells under an oil immersion lens with a micrometer eyepiece. When corpuscles are exposed to lead, the cell margin first becomes pale and then swelling occurs. Within an hour this is followed by shrinkage and a definite crenation of the surface. Then gradually the irregularities of these shrunken cells disappear and the outline becomes smooth and quite refractile to light. Maurel (285) also found that lead causes a marked deformity of cells. These phenomena, as well as others which also vary with time, suggest that the corpuscles react slowly with lead and that, under the temperature conditions of these experiments, equilibrium is established only after from two to five hours.

The interior of the cell. Since all these experiments have shown that lead affects the surface of the cell, the question arises whether there is any involvement of the interior of the corpuscle. That hemoglobin does not react with lead has already been pointed out. This is further corroborated by experiments carried out with Dr. A. V. Bock at the Massachusetts General Hospital, which showed that the gas exchange of "leaded" cells was apparently normal. That lead can penetrate the corpuscle and react with other substances

cannot be ruled out absolutely. However, the chemical findings which have been described previously indicate that the only cell constituent of those investigated in these experiments with which lead could unite is the inorganic phosphate. It could not combine with the organic phosphates, such as lecithin, unless they broke down and liberated phosphate in a simple state. Moreover, it is apparent that the first inorganic phosphate with which lead would come in contact would be that nearest the surface.

The explanation of the effect of lead. How can the changes in red blood cells caused by lead be best explained? Apparently they all depend upon the union of lead with inorganic phosphate and are largely surface phenomena. But diffusion experiments prove that the mere removal of ionized phosphate by lead is hardly sufficient cause for these effects. Despite the loss of phosphate by diffusion from red blood cells which stand in Ringer solution for several hours, such cells show no variation of the hypotonic saline curve from normal. The possibility that lead phosphate precipitates on the surface of the cell and exerts a modifying influence there must be considered. Phosphate probably unites with lead to form $Pb_3(PO_4)_2$. This may be precipitated in the membrane where its very presence may alter the colloidal properties. That it forms a complete coating on the outside of the cell, however, is hardly probable because of the very small amount of lead used. The possible surface area involved in this reaction cannot be determined because all cells are not equally affected by lead, and therefore calculations of the amount of lead available for each cell or of the amount of phosphate involved per unit of cell surface are not justified. A rough approximation, however, may be made which shows that the area of a red cell is at least of the order of magnitude of 10^{-7} sq. cm., while that of the lead molecules available for that cell is at most of the order of 10^{-12} sq. cm. Such a great difference makes the possibility of a superficial coating of the cells, even by a single molecular layer, highly improbable. Changes in the charge of the cell surface might explain the action of lead. This is suggested by phenomena like the disappearance of agglutination and the loss of stickiness and by rather marked differences between the agglutination of normal and "leaded" blood cells observed when tested with colloidal iron and arsenic. Macroscopic cata-

phoresis experiments however, showed no differences in migration between "leaded" and control cells.

The probable explanation of the effect of lead may rest on another basis. It has been shown that when lead chloride interacts with inorganic phosphate, free acid is liberated. This might be of importance in explaining the action of lead salts on red blood cells. Although buffer action would prevent any appreciable change in the hydrogen ion concentration of the blood as a whole, there might be a marked local temporary change at the surface of the cells. The objection may be raised that normally the buffers of the blood are quite adequate to take care of any acid produced. But it must be remembered that we are dealing here with a cell surface capable of local reactions and variations in permeability. Furthermore, the principal buffer, the phosphate, interacts with lead to form insoluble phosphate and free acid, so that its buffer action is diminished.

A series of experiments with collodion sacs illustrates this. When equal volumes of Ringer solution containing lead chloride (0.1 mgm. lead per cubic centimeter) and serum (diluted three times with Ringer solution) are mixed, no macroscopic precipitate forms. If, however, the serum alone is placed in a collodion sac and this is then suspended in the lead solution, a heavy precipitate forms at the membrane. If methyl red is added to each of these solutions, both remain alkaline to the indicator, but the membrane itself turns red. This demonstrates that acid forms locally. The same result is obtained if phosphate solution is substituted for serum.

These experiments with the collodion cells and the chemical reactions involved, therefore, indicate that the two reactions of lead on blood cells are precipitation of insoluble lead phosphate and production of acid. These local reactions may not be the only causes of colloidal change but are very important factors in producing all the phenomena described, for anything which changes the colloidal state of the cell surface probably affects its properties.

Summary. All the observations which have been presented fit into a complete and satisfactory explanation of the action of lead on blood. *In vitro* the exposure to a very small amount of lead greatly alters the surface of the red blood cells and causes them to shrink. Their consequent relative impermeability to water renders

them incapable of swelling as much as normal cells. This enables such cells to withstand a marked increase in their resistance to different osmotic surroundings, evidence for which is found in the abnormally small degree of hemolysis in salt solutions of very low concentration. This suggests that "leaded" cells should be really stronger and more durable than normal cells. But this is not the case. On the contrary, "leaded" cells are relatively short-lived and hemolyze readily as the result of slight trauma. Experiments with rabbits suffering from acute lead poisoning show that these phenomena also occur *in vivo*. In addition to causing these changes in permeability, lead also alters the physical properties of red blood cells so that they lose their normal stickiness and are no longer agglutinated by sera of the different iso-agglutinating groups. All these changes are evidence of surface alterations in the cell. The interior of the cell is not affected, i.e., the physiological properties of the hemoglobin remain normal. The chemical reactions which cause the physical changes in the cell are precipitation of insoluble lead phosphate and formation of acid. These cause the "leaded" red blood cell to change from an elastic, distensible sac to one which is contracted, relatively inelastic, and brittle. In such a condition the cell can so poorly withstand the trauma involved in circulation that this lack of resistance probably explains the marked destruction of peripheral blood in lead poisoning. Because of the loss of circulating red cells in this way, there is a compensatory regeneration of erythrocytes. Many of these young cells are affected by lead after they enter the circulation so that their basophilic substance is coagulated or clumped together and they give the characteristic granular appearance known as stippling. *In vitro* experiments indicate that lead may also have some inhibiting effect on the phagocytozing ability of polymorphonuclear leucocytes.

XII. EFFECT OF LEAD ON THE GERM CELLS

The effect of lead on the red blood cells may well be merely a striking example of an action which takes place in other cells of the body. The literature indicates that the germ cells are affected, but the data are generalized and only demonstrate that when either parent is subjected to the action of lead, the development of the young is impaired.

Constantin Paul (355) was the first to notice this. In 1860 he reported that lead poisoning of the mother or even of the father often caused stillbirth, and that the infant mortality of children born of such parents was particularly high during the first three years of life. This statement is based upon the observation of clinical cases, one of the most noteworthy of which is that of a woman who bore three normal children before her exposure to lead. After exposure she became pregnant ten times, but the only child which was born alive died at the age of five. In the same year Mattei (284) described one similar striking case. Others (25) (409) (367) (95) have since added confirmation to these reports by giving evidence that, after the ingestion of lead, stillbirths and miscarriages are frequent. Verhaeghe (476) gives statistics obtained from 90 lead workers, to whom 487 children were born after exposure. Although some of these workers had had healthy children before exposure, 107 of those conceived after absorption of lead were dead at birth, and 93 died before reaching two years of age.

In 1894 Porak (361) reported the results of experiments performed with female guinea pigs during experimental lead poisoning. He found that although abortion rarely occurred, many of the young were born dead or died soon after birth. Upon examination, lead proved to be present in their tissues, even a comparatively long time after birth. In spite of these results, however, clinical data seem to demonstrate that lead (especially in the form of diachylon ointment (376) (411) (184) (185) is an effective but, of course, dangerous abortifacient.

The effect of lead poisoning of the male upon development of the young has also been studied both clinically and experimentally.

In one case, Oui (348) reports that stillbirths occurred repeatedly while the father was suffering from lead poisoning; and in another a woman who had frequently had abortions when her husband was poisoned with lead, bore a healthy child by another man.

Lewin (260) states that lead causes abortion in animals as well as in man when either parent is poisoned, and that when absorption ceases, the deleterious effects of lead disappear.

Goadby (252) confirms this with evidence obtained from experiments on guinea pigs and also reports that lead injures the male germ cells. Further very careful investigations have been carried out by Weller (486) (487). He poisoned both male and female guinea pigs with lead before mating and found that when either was "leaded" the offspring were defi-

TABLE 36

Analysis of the tissues of offspring for stored lead after administration of lead acetate to mother

ANIMAL	DATES OF ADMINISTRATION OF LEAD	AMOUNT OF LEAD ACETATE GIVEN	DATE OF DELIVERY	NUMBER OF OFFSPRING	WEIGHT OF MATERIAL ANALYZED	LEAD FOUND	REMARKS
		grams			*grams*	*mgm.*	
Rabbit 294	November 14	0.581					
	November 28	0.581					
	December 10	0.830					
	December 14	0.830		8	50	0	Killed directly after birth
			December 17		40	0	
		2.822					
Cat 337	October 5 to March 1	7.7	September 14	4	70	0	Killed directly after birth.
					255	0	At end of two weeks. Mothers milk the only food. All kittens died
Rabbit 300	January 2	1.0					
	March 1	1.0	Animal died March 4		91.6	0.54	Uterus plus fetuses analyzed

nitely underweight and weak. The "leaded" males were not usually sterile, but when mated with a normal female the weight of their young was 18.5 per cent below normal. If the female was "leaded" and the male normal, the reduction in weight was somewhat less—about 15 per cent. In both cases the retardation of growth persisted after birth. Later experiments demonstrated that while plumbism in the male does not increase the number of stillbirths, intoxication of the female by lead does cause an increase which is out of all proportion to the severity of the poisoning. Cole and Bachhuber (84) have reported similar observations on rabbits and fowl. Paul's (355) investigations seem to indicate that lead injures only the germ cells formed during intoxication, for he reports that pregnancies were normal after exposure had ceased.

It is, therefore, clear that lead does not kill the germ cells, but only harms them so that the fetus either dies or is undersized. Injury is possibly caused in part by a reaction similar to that which occurs in red blood cells and in muscles. But that it may also result from a special susceptibility of the fetal tissues to the toxic action of lead has been suggested by the work of several investigators (361) (260) (293) (152) who report that lead is present in the tissues of the fetus when the mother is "leaded." The recent work of Blair Bell (32) is partly based upon such observations. In our few experiments, however, no lead was found in the young of "leaded" animals (table 36).

In explaining the premature deliveries and stillbirths which occur so characteristically during plumbism, still another mechanism is involved. Lead reacts with the smooth muscle of the uterus much as it does with the smooth muscle of the intestines during lead colic, and in this way probably causes abortion. The action of diachylon ointment is doubtless of this nature (see also page 124).

XIII. THE LEAD LINE

The most constant sign of plumbism and one which appears in no other condition, except possibly bismuth poisoning (237), is the so-called Burtonian or lead line in the gums. This has certain quite definite characteristics: It occurs typically in the gingiva near the border of the teeth; and is seen usually near decaying teeth, either in the gum or in the mucosa of the lip or cheek opposite these. More-

over, rapid caries seems to develop whenever a lead line appears near teeth apparently in good condition. That the pigmentation is not on the teeth themselves, may be easily demonstrated by inserting

Fig. 29. Microscopic Drawing Demonstrating Subepithelial Position of Lead Line

paper between the teeth and the gum. Furthermore, as the true lead line can never be rubbed off it must lie within the tissues and not be merely a surface deposit of pigment.

Microscopic examination shows that the line is composed of black, irregular amorphous granules which are entirely subepithelial. They occur in the interior and the walls of blood vessels, in connective tissue fixed cells, in macrophages, and especially in the papillae of connective tissue which run to the base of the epithelium (124). In figure 29 is shown the lead line as it typically appears under the microscope.

To explain the formation of this sign which is so indicative of lead absorption and so important in the diagnosis of lead poisoning, several theories have been advanced. All of these assume that the coloration in the tissues is caused by the interaction of dissolved lead and hydrogen sulphide which results in the formation of black lead sulphide. How the hydrogen sulphide is formed; how lead is brought to this region; how lead and hydrogen sulphide come into contact with each other; and by what chemical processes the lead sulphide is formed, are questions which must be answered before any explanation of the lead line can be complete.

Hydrogen sulphide is a product of putrefaction, especially of animal proteins. Thus there are two sources of this gas in the mouth: the debris of protein food caught between the teeth, and the decaying gums and teeth themselves. This explains one of the reasons for the clinical observation that a lead line rarely forms if teeth are cleaned regularly. In this connection it may be of interest to state that, even when severely poisoned rabbits, herbivorous animals, never have this lead line; while in cats, carnivora, it almost invariably appears very soon after administration of lead.

The mechanism by which lead is transported to the gums is less easily explained.

That lead may be directly absorbed by the mucosa of the mouth has been demonstrated by Blumgart (40). Constantin Paul (355) was able to produce a lead line even by the local application of lead to the gums. To answer Drisolle's (101) question whether the lead line may be a manifestation of a local action rather than of general absorption of lead, Paul painted the gums of four women with a solution of lead acetate. Their teeth were in perfect condition, and all other exposure to lead was prevented. After the first application of the solution, the teeth darkened and an incipient lead line could be seen; after the fourth this had become quite definite. It persisted for months, although there were no other signs of lead poisoning. This experiment could not be repeated on animals.

The suggestion has been made that lead is excreted by the salivary glands and accumulates in the mouth (365) (383) (400). If this actually occurs, lead could easily be reabsorbed by the mucosa. Schmidt (400), indeed, believed that the localization of the lead line is due to the special affinity of certain areas of the gum for lead excreted from the salivary glands. Although small quantities of lead have been found present in the salivary glands both by Pouchet (365) and in this laboratory in one cat, and a metallic taste is often complained of by individuals suffering from lead poisoning, evidence of such excretion has never been obtained. Moreover, the pigmentation frequently occurs in teeth which are not near the salivary ducts. Although lead may reach the gums directly under some conditions of exposure, this is probably neither the necessary nor usual process by which lead is localized in the gums. The appearance of a lead line in our cats after subcutaneous administration of lead is evidence that the lead may reach the gums either by excretion from the salivary glands or by the circulation. But the microscopic examination which shows that the black granules never appear scattered throughout the mucous membrane, as they would if lead were absorbed through the mucosa, but are always subepithelial in position, is a definite indication that lead is brought to the gums by the circulation.

Quite a different theory was advanced by Meillère (293) who believed that phagocytes carry lead to the gums for elimination. This view was based on the fact that many leucocytes containing particles of lead sulphide may be seen within the tissues of the gums. But the occurrence of such phagocytes probably indicates not that lead is transported to the gum, but rather that it is being removed from this region. In fact, similar phagocytosis occurs when insoluble particles of lead are removed from subcutaneous and pulmonary tissue or when mixed *in vitro* with leucocytes. This has been demonstrated by Carles (67) and by our experiments in which lead was administered by lung, and by the work of Fine (137).

In all probability lead, as the tertiary phosphate in colloidal solution, is carried to the gums, just as it is to all other parts of the body, by the blood. And wherever it meets the hydrogen sulphide which is formed in the mouth and diffuses through the mucosa a precipitate of lead sulphide is formed. The obvious objection to this idea is that

lead sulphide could hardly form if lead occurs in the blood as lead phosphate, a more insoluble salt. However, as H_2S accumulates from decaying material in the mouth, more lead must change from phosphate to sulphide in accordance with the law of mass action. This would be greatly accelerated if the reaction of the tissues was more acid than that of the blood for in an even slightly acid medium, tertiary lead phosphate is gradually converted to the di-lead salt which is about fifteen times more soluble than the sulphide.

There is evidence that changes occur in the gum before the lead line is grossly visible. In human beings (440) and also in cats, distinct localized hyperemia has been seen to precede the lead line. In this condition the gums are sore and bleed easily. Stephens believes that irritation causes enlargement of the papillae at the edge of the gum by engorgement of the vessels and that lead "finds its way" to this hyperemic region and "leaves its mark" on the gums. However, it seems more probable that the redness is merely an inflammatory reaction to the foreign particles of lead sulphide precipitated in the tissue. Microscopic examination verifies this opinion. In one of our cats the vessels in the inflamed gums appeared distended, and many red blood cells and polymorphonuclear leucocytes filled the submucosa, giving every evidence of marked acute inflammation. Scattered throughout this inflamed area black granules, presumably of lead sulphide, were seen. In another specimen from an animal with such irritated gums but with no visible lead line, the chemical test for lead was positive. From these observations the conclusion may be drawn that the deposition of lead salts in the gums begins before the lead line is grossly visible, and that the discoloration is evident only after the deposit becomes sufficiently great.

The possibility of an interrelation between the lead line and caries of the teeth is important practically. As early as 1870, Lewy (264) found that lead is deposited directly in the teeth just as in the other bones, and he believed that this might be a direct cause of caries. Recently lead has again been found in teeth as well as in the rest of the skeleton (page 75), but whether this is a factor in the development of caries or the lead line, is as yet unknown. The notoriously poor teeth of lead workers seem to indicate that the deposition of lead in the teeth may be important in causing decay. It might

be thought, however, that caries in the teeth of workmen is due to their unhygienic habits rather than to the action of lead. However, excellent evidence of the probable deleterious effect of lead on teeth is seen in cats who, under normal conditions, always have perfect teeth, but who develop rapid and marked caries when exposed to lead.

XIV. LEAD COLIC

Although not in itself of serious consequences, lead colic is the most painful and dreaded symptom of lead poisoning and presents a difficult diagnostic problem. The physiological mechanism and more particularly the direct cause of colic are as yet very little understood because of the scarcity of experimental data.

There is, however, general agreement that any condition of colic is due to marked constriction of the small intestine. This fact was first emphasized by Mackenzie (273), and post mortem examinations of animals dying from acute lead poisoning show that intense contraction of the gut actually occurs (337). The various explanations of the exact mechanism by which lead causes this spasm of the intestine fall into two general groups: (*a*) Lead may act on one of the nervous structures supplying the intestine; or (*b*) may directly affect the smooth muscle itself. A detailed presentation of each of these theories is hardly possible, for it involves a careful examination of the exact methods used in each case and would be essentially profitless. This is especially true of the ideas concerning the nervous origin of colic. Every possible nerve center and ganglion has been suggested as the site of lesion, usually with very little evidence. Among the structures mentioned are the sympathetic nerves (386) (217) (293); the coeliac ganglion (319); the vagus center (282), the submucous and myenteric plexus (274), and the ganglia in the intestinal wall (199). In addition, the literature contains many suggestions which can only be characterized as flights of fancy, such as reflex constriction due to myalgia of abdominal muscles (268) and irritation by hardened feces (464).

Before analyzing the various theories which have been advanced to explain lead colic, a consideration of the physiology of colic, of any origin, is desirable. If due to muscular constriction, colic must

result from either: (*a*) Stimulation of the vagus, through its center in the medulla, the nerve itself, or its endings; (*b*) inhibition of some part of the sympathetic nervous system; or (*c*) stimulation of muscle directly. A physiological observation, made by Cannon (64), should be mentioned here. He produced paralysis of the esophagus by sectioning the vagus and observed marked prolonged contraction of the cardiac sphincter of the stomach. He states that since this spasm occurs after vagotomy, it is independent of extrinsic innervation. The bearing of this phenomenon upon an explanation of lead colic depends upon the relation between loss of intestinal motility and spasm (page 165).

At this point mention should be made of a few of the more striking difficulties encountered in investigating lead colic before presenting the experimental and clinical evidence which has proved of value. The most baffling of these is to obtain from animals satisfactory data on such a subjective symptom as the pain of colic. Another and less well appreciated complication is the difference between experimental and actual conditions of exposure. Attempts which are frequently made to obtain strikingly positive results by intravenous administration of extremely large doses of lead salts, or by exposure to such very toxic organic compounds as lead tri-ethyl acetate, can, of course, hardly provide data from which to draw legitimate deductions as to conditions in human plumbism. From pathological studies of lead colic, just as of other manifestations of plumbism, unsatisfactory conclusions have been drawn on the basis of fragmentary and uncontrolled data.

With these facts in mind, some of the more important contributions to this question may be considered in detail.

Wassermann (481) poisoned cats with lead and examined the gastro-intestinal tract by x-ray. He found that the hyper-motility of the small intestine, with a disappearance of the usual beaded picture which first occurred, was followed by a broadening of the contents, which suggested loss of tone. In the large intestine the contraction rings and peristalsis were marked and this was accompanied by a delay in the excretion of the contents. Wassermann thought that colic is apparently associated with spasm of the small rather than of the large intestine and that during attacks the small intestine is contracted to the size of a thread.

X-ray examinations of one of our patients during a rather severe attack of colic gave results which seem to correspond to Wassermann's description. Definite areas of intestinal spasm were seen and the stomach which appeared hypertonic, exhibited exaggerated peristalsis. Six hours after the barium meal, the head of the barium column was held in the ileum; in twenty-four hours all the barium had accumulated in the cecum and ascending colon which appeared tremendously dilated, and none had passed the hepatic flexure. Over this area there was some tenderness. An enema given five days later showed no obstruction at the hepatic flexure, and another x-ray examination a week later showed nothing abnormal.

Although Wassermann made no microscopic examinations in his experiments, he attributes colic to sclerosis of the sympathetic ganglia, which has been reported by other investigators (see page 115). He believes this would permit unchecked vagus activity to cause intestinal spasm. To this view there are two objections: First, there is not sufficient evidence that lead causes a lesion in the sympathetic ganglia; and second, such a lesion, if it did occur, would impair the function of the ganglion permanently, whereas lead colic is a temporary condition from which recovery may be complete.

Somewhat similar conclusions were reached by Hirschfelder and his co-workers from experiments on intestinal peristalsis. They inserted a petri dish into the abdominal wall of rabbits as a window through which the intestine could be observed, and injected lead acetate (5 mgm. per kilo) into the circulation. This was followed immediately by the development of intense intestinal peristalsis which was unaffected by section of the spinal cord or vagus nerve but was abolished by the injection or application of nicotine or atropine. From these results they concluded that the spastic action of lead is due to its effect on preganglionic nerve fibers and not on muscle fibers. They also investigated the effects of nitrites on this tonic spasm and thought that they act directly on the smooth muscle of the intestine and do not relieve colic merely by general vaso-dilation. Several criticisms of this work should be made. Such immediate and acute intoxication is hardly comparable to that which occurs in life. Furthermore, such drugs as atropine and nicotine act on different structures and produce varying effects according to the strength of the dose. Atropine

may affect nerve plexuses or smooth muscle as well as act on the vagus, nicotine in small doses may stimulate the sympathetic instead of depressing the ganglia (86). Vagus section and cord excision, of course, have no effect on the activity of muscle fibers themselves or on myenteric reflexes.

Hanzlik (196) tried an ingenious experiment on the intact duck. By inserting a small rubber balloon into the crop he was able to record peristaltic contractions on a kymograph. They were more vigorous, rapid, and regular in lead poisoned ducks, and neither atropin nor epinethrin affected them, while chelidonin, which depresses smooth muscle independently of nerve endings, promptly inhibited the contractions. He, therefore, concludes that the augmentation of contraction due to lead was caused by direct stimulation of the smooth muscle.

In 1872 Kussmaul and Maier (243) suggested that an inflammation and ulceration of the gastro-intestinal tract caused by lead results in colic. This view, when first presented, was based on a single post mortem examination, but ten years later Maier (274) supported the idea by experiments with rabbits and guinea pigs. After the daily administration of lead salts in doses of 0.2 gram, degeneration of gland cells, hemorrhages, venous stasis, and sclerotic degeneration of the submucous and myenteric ganglion cells could always be seen in the intestine. While the afferent and efferent nerves appeared normal, the more central ganglia, such as the coeliac, often showed lesions. The severity of these changes suggests that the lead salt exercised a marked local action on the gastro-intestinal tract.

From experiments with isolated smooth muscle from the gastro-intestinal tract, the uterus, the blood vessels and ureters, Siccardi and Dozzi (431) were led to believe that lead acts to a great extent on the muscle fibers and not on the nerves alone.

These authors found that high concentrations of lead acetate increase the tone of isolated muscle strips but diminish the contraction waves. Upon removal of the lead, muscular activity returns to normal. Further support of the observations has also been given by Siccardi's (429) experiments with atropine, nitroglycerine, and nicotine. From his results Siccardi concluded that colic may be due either to the hypertonicity of intestinal muscles caused by the action of small doses of lead, or to reflex constriction following

the hypotonicity of muscle and cessation of rhythmicity which larger doses occasion. Unfortunately these experiments *in vitro* have little bearing on the action of lead within the body during chronic lead poisoning.

But that lead may exercise effects directly on smooth muscle has been shown by unpublished experiments with Miss Dorothea E. Smith (20). These were designed to determine whether lead acts on the myenteric nervous system or directly on the smooth muscle fibers themselves—a point not settled by Siccardi.

Strips of circular muscle containing no ganglion cells were prepared from the small intestine by the method of Gunn and Underhill (181) and the effects of lead salts on these were studied by the method of Magnus, as used by Cannon and de la Paz (64). The temperature of the apparatus was maintained at 37°C. and oxygen was continuously bubbled through the bathing Ringer solution. After normal contractions were recorded from all the strips, lead-Ringer mixtures were added to some of them. The rest were used as control preparations in the experiment. Immediately after the experiment a microscopic search for ganglion cells was made. Of twenty strips used in successful experiments, twelve were found to contain no demonstrable nerve cells, and on the five most satisfactory of these, lead was found to have the same effect that it does on longitudinal strips of smooth muscle and on strips containing nerve cells. Therefore, the assumption that lead acts *in vitro*, in large part at least, directly on the muscle fibers and not on the peripheral ganglia appears to be justified.

The effects of lead on smooth muscle as noted by Siccardi and confirmed in this laboratory by more than 110 observations are (*a*) inhibition of spontaneous contractions, and (*b*) an increase in tonicity. That these two phenomena are independent of each other is indicated in these experiments by the fact that in some cases small rapid contractions continue while the tonus rises markedly, while in others the rhythmical contractions cease although the tonus does not vary. Changes caused by 1 mgm. of lead acetate per 100 cc. of Ringer solution, or 1 part per 100,000, are not always permanent, but may persist for a short time and be followed by gradual recovery of the muscle, even when the lead is not removed. In tests of the relative toxicity of lead acetate, lead chloride and lead nitrate, no distinct differences between these salts were observed. The addition of serum albumin did not markedly alter the results, but changes in the

hydrogen ion concentration caused striking variations. At pH 6.5, as would be expected, lead exercised much less effect than at pH 4.8.

The action of lead on smooth muscle as described in these experiments is made very significant by the clinical observation that lead colic is almost invariably preceded and accompanied by obstinate constipation. Tanquerel des Planches (453) lays great stress on this feature of plumbism in the twelve hundred cases which he reported. Also Heubel (213) and Meillère (293) were both convinced of the distinct connection between colic and constipation, and Oliver (337) and Legge and Goadby (252) were impressed with the relationship. Furthermore, none of our patients developed severe colic even when excreting relatively large amounts of lead. This was probably due to the fact that constipation is prevented as part of a hospital regime.

Thus, there are two outstanding features of the mechanism involved in lead colic: (*a*) Lead inhibits the motility of smooth muscle while it increases the tone; and (*b*) clinically colic is preceded by obstinate constipation. As yet, sufficient data for a definite physiological explanation of lead colic have not been collected, but it is quite apparent that lead does not exercise a selective action on any of the nervous structures of the gastro-intestinal tract. From a survey of the meager experimental data and the rather definite clinical observations, the most reasonable suggestion to explain lead colic is that lead acts directly on the smooth muscle of the intestine, producing increased tone and loss of motility, and therefore constipation results. The griping pain of colic may be due to a reflex constriction which approaches such an amotile, hypertonic area, a condition similar to that found in intestinal obstruction (63b).

XV. NERVOUS REACTIONS

Lead palsy. Paralysis is the most incapacitating manifestation of lead poisoning and is especially distressing because of its chronicity and because the muscles affected are usually those most used in routine work. As almost all investigations of this condition have been pathological and clinical in nature and have consisted of careful general and microscopic examination of the neuro-muscular apparatus, much detailed information about the late gross and minute lesions of palsy

has been collected. From this, attempts have been made to deduce the process by which lead causes paralysis. In order to evaluate these studies, it may be well to give an orderly and systematic summary of the various theories which have been offered and to mention some of their proponents. For convenience in discussion, the pathological, clinical and physiological aspects of lead palsy will be considered separately.

Pathology. From a study of the morbid anatomy and histology of patients with lead palsy, and from the observations on experimental animals, theories of the mechanism of lead paralysis have been evolved which locate the primary lesions in every part of the neuro-muscular system.

As long ago as 1879 Friedländer (149) considered muscular changes of first importance and described a degenerative myositis in which the sarcolemma was primarily involved and which was characterized by marked fibrous tissue replacement. He believed that the involvement of nerves supplying the atrophied and degenerated muscles were secondary to a primary muscle injury. Kast (231) was the only other investigator to commit himself definitely in favor of this view until Messing (301) pointed out quite clearly that careful microscopic examination always demonstrated that the lesions in the muscle are more marked than in any other part of the neuro-muscular apparatus. Since the lesion is not a simple atrophy but really a specific inflammatory process, he concluded that lead must act directly on muscle and not merely interrupt trophic fibers by causing lesions in peripheral nerves.

Other investigators have suggested that muscle plays only a secondary rôle in the etiology of lead palsy, and that lead acts primarily on the vascular system (218) (364) (381) (252).

According to them, lead directly injures the vessel walls so that they rupture with relative ease during muscular activity in both nerve and muscle. Messing also believed that the essential lesion in muscle was vascular, but in his careful examination he found that the blood vessels were affected only in the muscles involved—a fact which throws doubt on the general vascular action of lead, and supports the view that lead acts directly upon muscle.

Most pathologists, however, consider that the primary lesion of lead paralysis is in the nervous system. The idea that it is in the

spinal cord has many supporters (120) (380) (91) (313) (335) (499) (360) (443) (479) (381) (341), most of whom base their opinion on the appearance of distinct degenerative lesions in the anterior horn cells; while another large group of investigators consider changes in the peripheral nerves to be of fundamental importance (245) (490) (342) (92) (172) (77) (265), and describe a distinct Wallerian degeneration in the nerves running to the paralyzed muscles. Déjérine-Klumpke (92) emphasizes the fact, which Gombault (172) first pointed out, that the lesion is distinctly peri-axial.

The conception, however, that the lesion of lead paralysis is essentially complex has been most generally accepted, even by those who believe that lead acts directly upon a particular part of the neurone (36) (406) (371) (478) (357) (138) (435) (173) (494) (89). According to this view, lead affects that part of the neurone which for the time being is rendered most susceptible by its functional condition, i.e., the *locus minoris resistentiae* (379) (173). Recently Hyslop and Kraus (224) have summarized the pathology of lead palsy and have concluded that the lesion is probably a neuronitis which may be manifested in any part of the neuro-muscular apparatus. In evaluating this pathological work, however, it is important to realize that all examinations were made after death, and in many cases only after palsy had persisted for several years. In most of these investigations the attention of the pathologist was so focused on some special part of the neuro-muscular system that the rest was neglected, and usually it was the muscle which was ignored. At best, post mortem examinations can give but a slight suggestion of the conditions which require dynamic methods of investigation. A satisfactory explanation, therefore, cannot be derived from pathological examinations alone.

Clinical observations. Quite early, investigators attempted to determine the pathogenesis of palsy by careful clinical examinations. One of the first observations, that the extensors of the forearm are generally involved in peripheral palsy, suggested the idea that lead may be absorbed through the skin and thus injure the muscle by direct contact (280) (108) (276) (392). As soon as knowledge of the absorption of lead accumulated, however, this idea became untenable. Déjérine (91), Remak (380), and von Monakow (313) believed that

only central lesions could cause paralysis of functionally correlated muscles and explain the symmetrical character of the lesions. But more recent observations have shown that the peculiar localization of palsy has an altogether different explanation and that the symmetry of the lesions is over emphasized. There is some clinical evidence which points toward the muscle itself as the site of the action of lead. A reaction of degeneration is obtained from direct stimulation of paralyzed muscles as well as of nerve, but that this may merely indicate a secondary muscular atrophy following a nerve lesion must be kept in mind. The susceptibility to fatigue and the general weakness of patients suffering from plumbism have long been recognized by clinicians. The occurrence of paralysis in fatigued muscles is said to have been first pointed out by Meyer (304) in 1854, and definitely described by Moebius (312) in 1886. Through the work of Edinger (105) this observation has been made the most important single clinical contribution to the explanation of palsy and has been accepted by almost every student of plumbism (484) (455) 337) (168). In an attempt to explain this observation, Hitzig) (218) in 1868 suggested that since the region with the greatest blood supply must receive the most lead, the fatigued muscles in which the circulation is greatly increased are necessarily exposed to the largest quantity of lead. Such an explanation, however, gives no clue to the actual mechanism by which lead acts on muscle tissue. It is evident, therefore, that this interrelationship of fatigue and the development of lead palsy can be solved only by chemical and physiological investigation.

Physiology. The importance of the action of lead on the physiology of the neuro-muscular system was apparent to some of the earlier investigators, and in fact as long ago as 1877 Muson (325) is reported to have demonstrated palsy in the hind limbs of frogs after immersing the animals in water containing lead. He found no lesion in either nerve or muscle. Since that time some interesting and suggestive experiments have been performed which constitute the basis of conflicting theories. These are fully discussed in an article by Reznikoff and Aub (385). Harnack's results (199) which were partly due to the ethyl group of the organic compound he used, led him to believe that lead palsy is caused by direct injury to the muscles; and Cash's un-

controlled experiments (71) confirmed his observation that after exposure to lead the relaxation time of muscle is prolonged. Mellon (296) also obtained data which seem to support the theory that lead produces a definite functional lesion in muscle. Dozzi (100) on the other hand, suggested that lead might affect isolated nerve as well as muscle and obtained results which apparently strengthened his theory; but too much reliance must not be placed upon these observations because he used very concentrated lead solutions of unknown acidity which contained no other salts. This brief summary indicates that our knowledge of the physiological action of lead on the neuro-muscular system is very slight. Such ignorance could in the past be accounted for partly by lack of knowledge of the physiology and chemistry of muscle, which recent investigation has removed to a considerable extent (139) (140) (141) (142) (144) (219) (216) (305) (115) (116); and partly by a lack of accurate chemical and physiological knowledge of the action of lead within the body.

Some of our investigations have suggested a means of studying the physiological aspect of lead palsy. In experiments dealing with the effect of lead on red blood cells (15) (16) (17), it was found that lead so alters the surface of the corpuscle that its permeability to water is changed. The question arises, therefore, as to whether lead may not act on the surface of muscle in a similar manner and thus change its permeability.

Of the various methods of studying the passage of substances through the surface of muscle (142) (219) (216) (305) that devised by Embden (116) is the most satisfactory for our purpose. It is based upon his observation that an increase in the diffusion of inorganic phosphate from muscle is due to an increase in the permeability of the cell membrane. Because of the ease of determining the quantity of inorganic phosphate in solution we have employed this method in a study of the effect of lead on the permeability of the surviving muscle of the frog (385).

In our experiments, isolated muscles from frogs were placed in beakers containing Ringer solution. The quantity of inorganic phosphate which diffused into the bathing fluid was determined hourly until a low and practically constant value was obtained. One of the muscles was then placed in Ringer solution containing lead (0.05 mgm. Pb per cubic centimeter) and

allowed to stand for one hour, while the other was kept as a control in regular Ringer solution. Both muscles were then transferred hourly to fresh Ringer solution and the inorganic phosphate content of corresponding solutions from the control and "leaded" muscles was determined. The analysis for inorganic phosphate was carried out by a modification of the Bell-Doisy blood method in which the protein precipitation stage was omitted, and the entire quantity of fluid was compared with standards prepared simultaneously with the unknown. Nessler tubes were substituted for a colorimeter, in order to decrease the error in matching the very faint colors produced by the small quantities of phosphate. From these results, a curve of phosphate diffusion was obtained for each muscle and any difference caused by the action of lead could be determined.

Thirty-four out of thirty-five experiments showed quite definitely that the diffusion of inorganic phosphates (expressed as milligrams of P per gram of muscle per hour) increased markedly after "leading." Control muscles underwent no such change, except for a slight increase following stimulation in some experiments. Their diffusion followed the same course as that in Fletcher's CO_2 experiments, which was characterized by an immediate rapid decline succeeded by a very gradual decrease in the rate of diffusion. A summary of the results of all the positive experiments shows that after exposure to lead the average rate of diffusion rose 320 per cent above the lowest value. In only two cases was the increase less than 100 per cent while in nine it was between 100 and 200 per cent; in ten, between 200 and 300 per cent; and in two experiments more than 1000 per cent. Figure 30 illustrates one of the most striking experiments and figure 31 is a composite curve of all the 34 positive results. After the muscles were removed from the solution of lead, there was considerable variation in the length of time which elapsed before the diffusion of phosphate became maximal, but as a rule this was about three hours. The duration of maximal diffusion was usually only one to two hours, but in most cases the rate did not return to the low original value during the experiment.

Other factors than exposure to lead such as stimulation, injury, or rigor may alter the rate of phosphate diffusion. Control experiments showed that injury was a negligible factor in this work and that stimulation produced only a very slight rise in some cases. Rigor was avoided by providing a constant supply of oxygen and by

preventing undue fatigue. Although the relationship between rigor and the muscular changes caused by lead cannot be considered in detail here, it will be of interest to remember that rigor is supposed to be due to the acid products of fatigue. Examination of muscles after exposure to lead demonstrates that they are shrunken, of rubbery consistency, and lusterless—in fact, quite comparable in appearance to muscles in rigor.

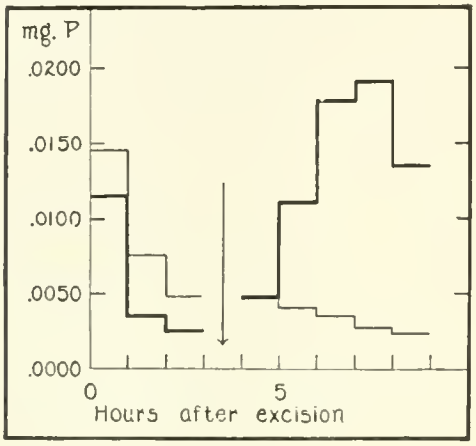


FIG. 30

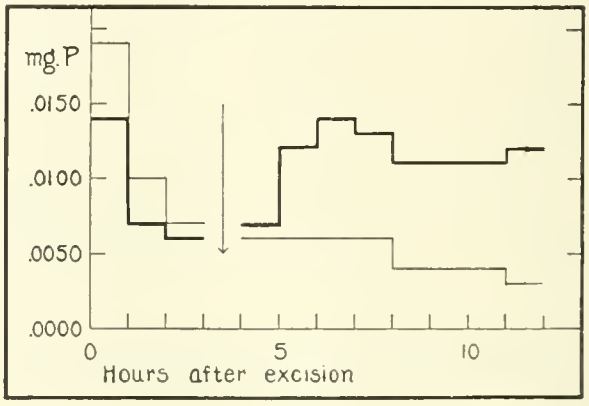


FIG. 31

FIG. 30. CHART SHOWING EFFECT OF LEAD UPON THE RATE OF DIFFUSION OF INORGANIC PHOSPHATES FROM MUSCLE

The heavy line represents the rate from "leaded" muscle; the light line that from the control muscle. The arrow represents period of exposure to lead (0.05 mgm. Pb as $PbCl_2$ per cubic centimeter Ringer solution).

FIG. 31. COMPOSITE CHART SHOWING EFFECT OF LEAD UPON THE RATE OF DIFFUSION OF INORGANIC PHOSPHATES FROM MUSCLE

The heavy line represents the rate from the muscle exposed to lead solution; the light line that from the control. The arrow represents period of exposure to lead (0.05 mgm. Pb as $PbCl_2$ per cubic centimeter Ringer solution).

Some other observations merit consideration. If a "leaded" muscle is treated with ammonium sulphide and examined under the microscope, all the lead appears on the surface and none has penetrated into the muscle. Another fact is also of interest in this connection. When a muscle is exposed to lead there is a change in the reaction of the surrounding solution, in one case from pH 6.5 to 4.8, in a second experiment to pH 5.5, and in a third to pH 6.1. Such an increase in acidity agrees with the results of our work on the effect

of lead on red blood cells, which show that soluble lead salts unite with the inorganic phosphate to form insoluble lead phosphate with the liberation of free acid. To determine the effect of acid on the permeability to inorganic phosphate, acid Ringer solution was added to muscles. In one case, in which the pH of the Ringer solution was 4.5, the permeability of the treated muscle remained practically like that of the control, but in two experiments in which the pH of the Ringer solution was 3.5, the rate of phosphate diffusion increased markedly at once. The maximum increase was very brief, but throughout the experiment the rate of diffusion remained constant and did not decrease as did that of the control. In one case it remained twice as high as the original rate before exposure to acid. The maximum increase in diffusion due to acid was 140 per cent in one of these experiments and in the other 520 per cent. That this type of experiment is not comparable to those in which the muscle is exposed to a lead salt, must be remembered, for in the latter case acid is produced in the tissue of the muscle and the buffer phosphates must be diminished by their interaction with lead. But these acid experiments offer further evidence that when lead salts act on isolated muscle the reaction which probably occurs is similar to that with red blood cells, and as a result there is a marked change in the permeability to inorganic phosphate. That this is due to an alteration of the surface and not to the break-down of increased amounts of organic phosphate within the muscle, is indicated by the increased rate of diffusion after removal of the muscle from the lead solution and by the persistence of this increase for several hours, usually throughout the experiment.

Such a striking change in the permeability of the surface of muscle to inorganic phosphate suggests that lead may have a demonstrable effect on the activity of isolated muscles. To determine this, experiments were performed on nerve-muscle preparations (sciatic-gastrocnemius) in specially constructed chambers which are fully described in a detailed account of the experiments (385). These permitted simultaneous stimulation of either both nerves or both muscles since the electrodes were arranged in series, and also allowed the Ringer solution to be drawn off for analysis of inorganic phosphate.

Thirteen experiments were performed with tetanic stimulation. In five of these no difference could be seen in the rate of onset of fatigue

in the control and "leaded" muscles, probably because the great amount of work done fatigued both muscles very rapidly and caused rigor. In no case, however, did the control fatigue more rapidly than the "leaded" muscle. In eight experiments in which the control muscle remained in good condition, the "leaded" muscle fatigued very

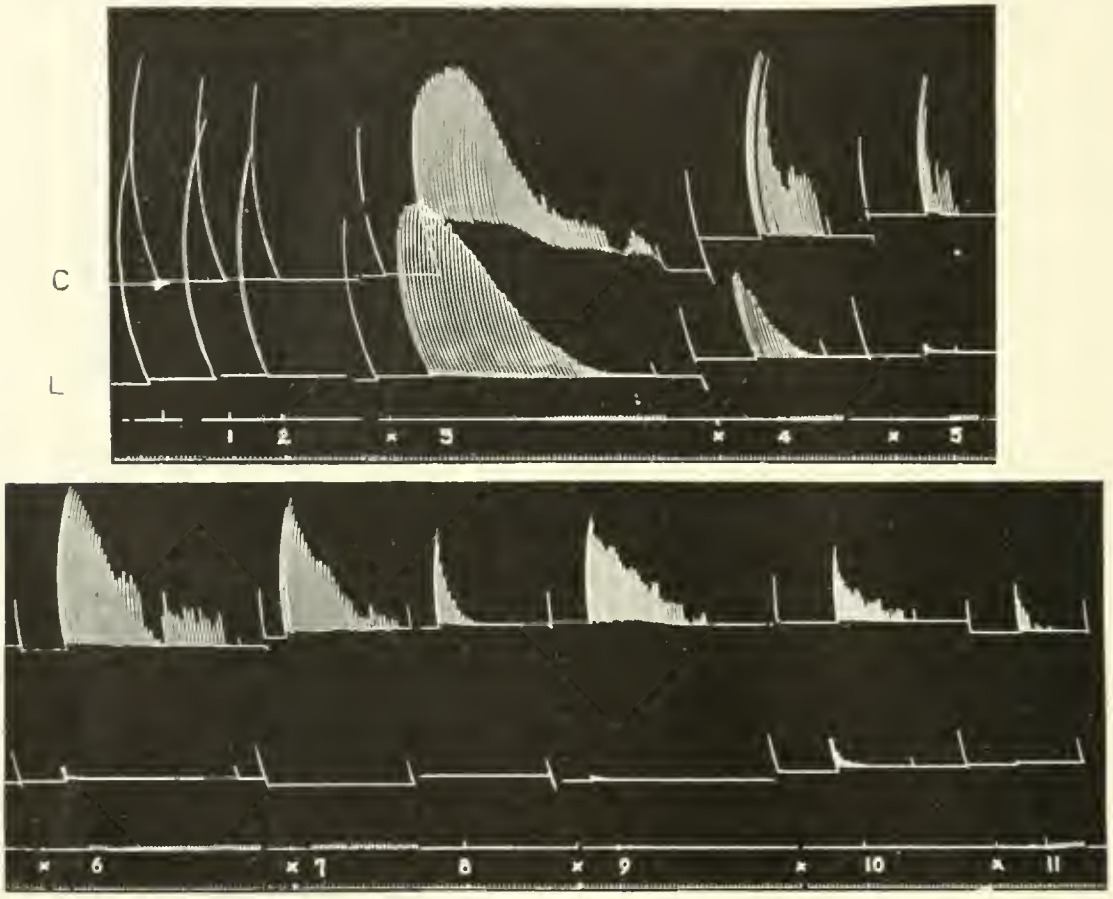


FIG. 32. GRAPHIC RECORD OF MUSCULAR RESPONSE TO INTERMITTENT TETANIC STIMULATION BEFORE AND AFTER "LEADING"

C is the tracing made by the control muscle; *L* is the tracing made by the "leaded" muscle (0.05 mgm. Pb as $PbCl_2$ per cubic centimeter Ringer solution). The figures indicate the periods of stimulation; the crosses represent readjustment of the after loading of the muscles.

rapidly and usually did not recover. Figure 32 illustrates one of these in which the muscle was stimulated directly and the rate of phosphate diffusion was determined hourly (fig. 33). This experiment demonstrated several interesting facts. The "leaded" muscle fatigued much more rapidly than the control and failed to recover. The excellent initial contractions obtained after removal from the

lead-Ringer solution demonstrate that the action of lead is manifested only after muscular activity. Comparison of the diffusion curve and muscle tracing shows that the greatest quantity of phosphate diffuses when the muscle is most fatigued during the first, second, and especially the third hour after exposure to lead.

Because tetanic stimulation seemed to fatigue control muscles so readily, a series of experiments (ten) was performed with make and break stimulation. These tests showed very distinctly that after an

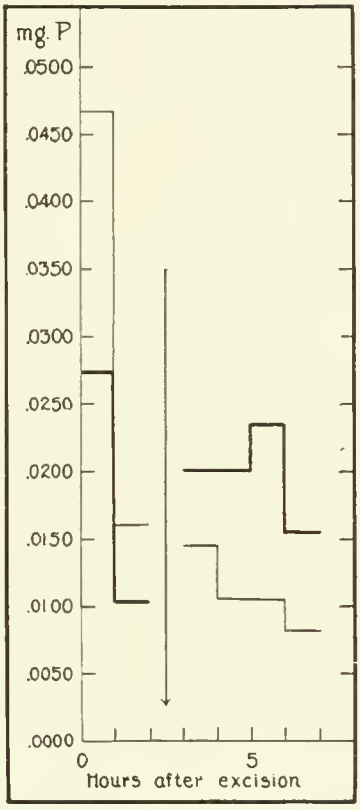


FIG. 33. CHART SHOWING VARIATIONS OF THE RATE OF INORGANIC PHOSPHATE DIFFUSION FROM NORMAL AND "LEADED" MUSCLES DURING TETANIC STIMULATION

The heavy line represents the rate from the "leaded" muscle (0.05 mgm. Pb as $PbCl_2$ per cubic centimeter Ringer solution); the light line that from the control muscle.

initial period of sustained uniform contraction, the "leaded" muscle fatigues more rapidly and completely, and recovers with much greater difficulty than the control in every case (fig. 34). From these investigations the conclusion may be drawn, therefore, that lead interferes with the function of isolated muscle very markedly. Stimulation produces fatigue much more rapidly after exposure to lead; while recovery, difficult at best, in most cases does not occur at all.

The next question to be considered is the effect of lead on nerve. Because of the clinical designation of lead palsy as a peripheral neuritis as well as the occurrence of lesions in peripheral nerves, the impression is prevalent that lead acts specifically on peripheral nerve. The

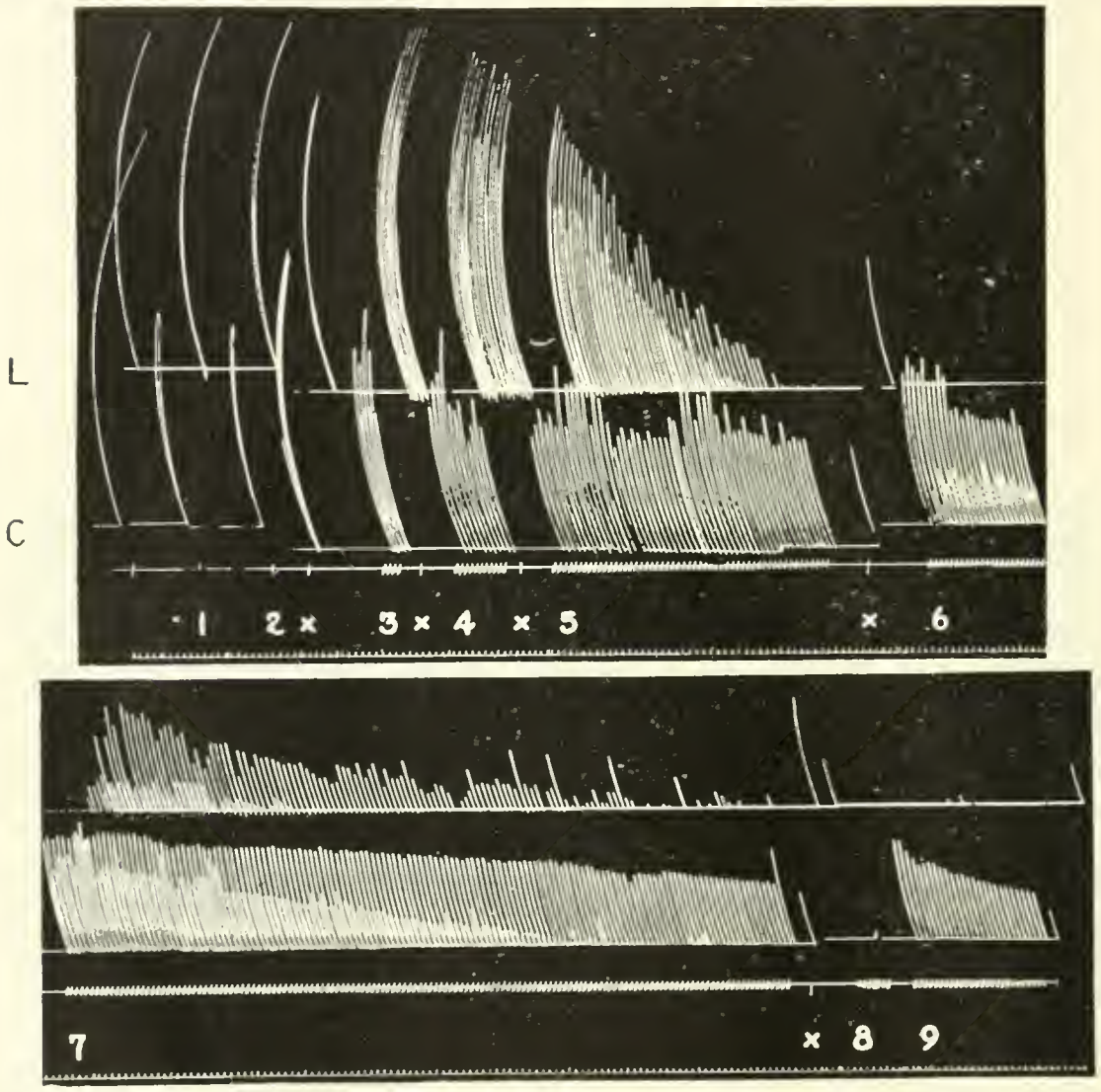


FIG. 34. GRAPHIC RECORD OF MUSCULAR RESPONSE TO MAKE AND BREAK STIMULATION

L is the tracing made by the "leaded" muscle (0.05 mgm. Pb as $PbCl_2$ per cubic centimeter Ringer solution); *C* that of the control.

only experimental study of this problem reported in the literature was made by Dozzi (100) which has already been criticized.

The action of lead on isolated nerve has been studied in this laboratory (385). As judged by muscular response, the conductivity

of the "leaded" nerve was exactly the same as that of the control even after exposure to concentrations of lead as high as 0.46 mgm. per cubic centimeter—the largest quantity soluble in Ringer solution of pH 6.5. Therefore, as far as such experiments can determine, lead produces no decrement in nerve conduction. To check these results, an attempt was made to determine by the string galvanometer the action currents of both nerve and muscle as a delicate index of function. In one of a pair of nerve-muscle preparations the nerve was exposed to lead, in the other the muscle. One experiment demonstrated quite distinctly that after fatigue the action current of the "leaded" muscle diminished rapidly and soon disappeared entirely, while that of the control muscle returned to normal within a short while and persisted until the end of the experiment. Similar undiminished action currents were obtained from both normal and "leaded" nerves. Thus, the distinct inhibitory action of lead on isolated muscle and the lack of deleterious action on nerve is confirmed.

In the explanation of lead palsy, as it occurs in life, the experiments described serve merely as preliminary and suggestive observations which must be correlated with the production of palsy in living animals if they are to have any important practical significance. The ample evidence that in life lead paralysis develops in muscles which are fatigued (304) (312) (105) (484) (455) (337) links the problem of lead palsy with the chemistry of muscular fatigue and suggests a method of investigation in intact animals. Consequently, a series of experiments was carried out in an attempt to obtain explanatory data. In order to produce unilateral fatigue, weights were attached to the dorsal surface of the right forepaw of severely "leaded" and control cats, and the animals were exercised daily in a revolving drum. Marked fatigue was thus obtained in the extensor muscles of the right forepaw by constant raising of the weights. When a distinct difference between the weighted limb and the other became apparent, the animal was anesthetized and the thresholds of these muscles and their nerves were determined. The results of these mammalian experiments are very striking. After the administration of lead all the cats lost weight very rapidly and within one or two weeks showed distinct lead lines and became fatigued by exercise very easily. The weighted foot showed distinct signs of weakness after about two weeks

of exercise. This was manifested by difficulty in extending the foot when the weight had been removed for twenty-four hours, and was especially marked if the animal was held by its neck and permitted

FIG. 35. PICTURE OF CAT SHOWING PALSY OF RIGHT FOREPAW

to reach for the top of a window ledge. Figure 35 illustrates the flexed position of the right forepaw in one of the "leaded" cats. Some of the animals limped decidedly in walking. When the weights were still attached, the contrast between the "leaded" and control animals

was even more striking. Those suffering from plumbism could not lift the weighted paw without great effort and then only with the aid of the upper leg muscles, while the control animals lifted both paws with equal ease. After running in the drum, these differences were accentuated. The "leaded" cats tired much more quickly, and to a greater degree than did the normal animals, and exhibited a very striking fatigue of the weighted limb, which usually completely prevented extension of the paw. They took shorter steps with the weighted limb while running, and seemed to lean toward the left. This picture fits almost all of our "leaded" cats in practically every respect.

One of the most striking features of these experiments is the similarity between the threshold of the unweighted muscles of the "leaded" animals and that of both unweighted and weighted muscles of the controls when stimulated by nerve. The threshold of the weighted muscle of the "leaded" animals to stimulation through nerve was always very much higher whenever the muscle was distinctly weaker than that of the opposite side (four cases). The relative difference between the two sides was also apparent with direct muscular stimulation in most of the cases tested.

Proper appreciation of these experiments requires a brief consideration of the chemical relationship between lead in the body and the products of muscular activity. Lead is deposited in the bones as insoluble lead phosphate (see page 43) but is mobilized by various changes in metabolism. As is discussed elsewhere (section IV), lead probably acts in this way because the phosphate is very soluble in acids, especially in lactic acid which is liberated in large amounts during muscular activity, particularly during fatiguing exercise (387) (26). Therefore, when lead phosphate circulates through muscle undergoing vigorous exercise, it must be dissolved to a considerable extent by the lactic acid and thus be converted to lead lactate.

If such a soluble lead salt as the lactate comes into contact with a cell, it can unite with the inorganic phosphate present at the surface (129) and form insoluble lead phosphate and free lactic acid. In work already reported (17) it was suggested that the precipitation of this insoluble lead salt, which is accompanied by the liberation of free acid and the removal of buffer phosphate, changes the colloidal

state and the properties of the cell surface. There is considerable evidence that this occurs in muscle. Clinical observation has established the fact that the muscles paralyzed are those which are most used. As far as is known, only muscle and not nerve becomes fatigued; and experiments *in vitro* have demonstrated that lead does not interfere with the function of isolated nerve, whereas it does affect muscle markedly. This change in muscle function is preceded by an alteration of the surface permeability, and it is therefore very probable that lead acts upon muscle just as upon red blood cells, by changing the permeability of the surface.

Thus these considerations afford a possible explanation of the development of lead palsy. Lead is transported by the blood as an insoluble phosphate in colloidal state. In regions of muscular activity this is dissolved by the excess lactic acid which diffuses from fatigued muscle cells and is converted into lead lactate. As the soluble lactate comes into contact with inorganic phosphate at the surface of muscle cells, the lead is reprecipitated as insoluble phosphate—a reaction dependent upon the relative concentrations of lactate and phosphate. This work, therefore, suggests that the physiological lesion of lead palsy is in the muscle itself and that those muscles which are fatigued are most susceptible to lead paralysis. Furthermore, it suggests that the susceptibility to lead palsy depends on the chemical reactions between lead and the metabolic products formed during muscular activity.

The question of how to correlate these results with the pathological and clinical observations naturally arises. There is no doubt that distinct lesions occur in the peripheral nerves and spinal cord in lead palsy. That a lesion in a neurone is followed by peripheral degeneration is well known and is the foundation for the opinion that palsy is essentially of central origin. But the occurrence of distinct primary lesions other than atrophy in the muscles and simple Wallerian degeneration in the peripheral parts of the nerves, demands some explanation of primary peripheral injury and secondary central degeneration. This question troubled even the early workers.

Putnam (371) thought that central changes might be secondary to peripheral injury although he offered no explanation, and indeed this has

been suggested by all investigators who believe that lead paralysis is essentially a neuronitis. According to Meillère (293), the most vulnerable part of the neurone is affected by lead, and a lesion once formed may spread either centrifugally or centripetally. Although Remak (380) considered palsy to be of central origin, he stated that for the most part lesions are present in the muscles and in the peripheral parts of the nerves and that they become less marked centrally. This was expressed even more positively by Legge and Goadby (252) who said that the more recent the palsy the more severe are the peripheral lesions, and that as the condition becomes more chronic the central lesions are more marked. Although the exact mechanism by which peripheral injury causes central lesions is not known, suggestive work by Orr and Rows (345) possibly throws some light on the subject. By experiments with tetanus toxin, bacteria, and dyes, they demonstrated a lymphogenous drainage from the periphery up the nerve to the cord, and the lesions which they described were in the adventitia around the veins and capillaries; and seemed very much like the lesions characteristic of the early stages of various forms of peripheral neuritis. Walshe (480) explains the mechanism of diphtherial paralysis on the basis of these investigations. This work, however, is merely suggestive, and further investigation is necessary before such an explanation can be applied to lead palsy.

In conclusion it must be pointed out that it is impossible to explain all the phenomena associated with lead paralysis. Although under ordinary conditions lead may have no specific predilection for any tissues except bone, it may produce a very marked effect upon certain tissues when their physiological conditions change. Thus, the action of lead on quiescent and on fatigued muscle may be very different. In the light of the more recent work on the chemistry of muscular activity and of lead within the body, it seems probable that the physiological lesion of lead palsy is in muscle. The occurrence of pathological changes in nerve and spinal cord to which so much importance has been attributed by older workers, may, according to more recent views, simply result from peripheral injury. Whatever remote and indirect lesions lead may produce, it is probable that in lead palsy there is a close chemical and physiological relationship between the action of lead and muscular activity, which may have great significance in many clinical and therapeutic problems.

XVI. MENINGO-ENCEPHALOPATHY

Perhaps the most dramatic manifestation of lead poisoning and at the same time one which is of the most serious prognostic import is the development of acute mental changes. This condition, known as encephalopathy, was described as early as 1837 by Grisolle (178). Many theories, which point to various structures as the site of lesions, have been advanced to explain the etiology of the symptoms. Encephalopathy has been ascribed to (*a*) cerebral arteritis (463), (*b*) cerebral arteriosclerosis (222), (*c*) cerebral anemia (390) (213) (293) (225) (300), (*d*) punctate hemorrhages due to arterial and venous degeneration (252), (*e*) uremic manifestations caused by the action of lead on the kidneys (88), (*f*) the direct action of lead on the cortical cells (see Pathology), and (*g*) to meningeal lesions (315) (316) (332) (50) (466) (203).

Analysis of these theories shows that some may be quite readily discarded. The isolated observations of arteritis and arteriosclerosis have never been substantiated. The suggestion of other vascular changes as causative factors has more foundation. Some evidence has been presented to support the idea that cerebral anemia, due to spasm of the vessels, is the basis of encephalopathy. Siccardi and Dozzi (431) found that isolated arteries shrink in solutions of lead salts; but it is hardly probable that lead can thus affect the blood vessels *in vivo* (see Pathology). Although lead is generally considered a vasoconstrictor and some believe that clinical conditions may be explained by this action (206), it has never been definitely shown that lead causes a general rise of blood pressure (131). Mason (282) observed that after the injection of lead tri-ethyl acetate into animals the immediate fall of blood pressure was followed by a marked rise. But it must be remembered that lead tri-ethyl acetate itself has a toxic action quite distinct from that of lead. Furthermore, Page (350) believed that in workers who have been exposed to lead, hypertension usually is accompanied by some renal impairment. The typical behavior of patients suffering from encephalopathy is another objection to the theory that cerebral anemia is responsible for the condition. Since anemia always causes unconsciousness, it cannot be the basis of sustained excitability, which is so frequent during this manifestation

of plumbism as to be almost characteristic. General secondary anemia, however, is a common sign of lead poisoning (see page 188) and might account for the punctate hemorrhages to which Legge and Goadby attribute lead encephalopathy. Their hypothesis which is based on a few experimental observations and principally on a clinical report by Mott has already been discussed in the section on Pathology, and no further comment is required to indicate its inapplicability to the mental symptoms of plumbism.

That encephalopathy may be of uremic origin, has never been substantiated by renal examination (see Pathology). The pathological picture, in spite of clinical opinion (493), blood pressure measurements, urine analyses, and blood chemistry determinations has rarely pointed to the kidney as the immediate etiological factor in encephalopathy (390). That lead acts directly on the cortical cells is suggested by the presence of lead, although in very small amounts, in the brain. But the relatively slight damage seen in pathological examinations induced Meillère (293) to point out that "the violence of the symptoms observed contrasts singularly with the prompt re-establishment of apparent integrity." He believed that aside from some evidence of anemia in the gray matter no other microscopic or macroscopic lesions exist.

The idea that lead encephalopathy may be principally a meningitis or meningopathy is of quite recent origin and is based on three separate pieces of evidence: The pathological observations of Hassin (203); the experiments of Camus (61); and the changes of the cerebro-spinal fluid reported by Mosny and Malloizel, Norton, Boveri, and Troisier.

At post mortem examination, Hassin always observed a marked proliferative meningitis and in acute cases some round cell infiltration of the meninges. The lesions in the brain, however, were relatively inconstant and slight, and consisted for the most part of vascular proliferation extending from the meninges. In this connection it is interesting to note that almost all pathologists before Hassin had focused their attention upon the brain and had ignored the meninges.

Camus is one of the few who have studied lead encephalopathy experimentally. He injected lead into the subarachnoid space of a dog by cistern puncture and within two or three days symptoms of encephalopathy ensued—excitability, "hallucinations," and con-

vulsions just before death. If he injected lead directly into the brain substance, no symptoms developed and only encysted necrosis of small areas of the brain resulted. Neither did the mere administration of lead intravenously produce any mental symptoms; but of very great interest is the fact that simultaneous injection of a non-toxic aseptic irritant into the subarachnoid space and of lead by vein resulted in encephalopathy. According to Camus, lead is transported as particles of lead albuminate by the leucocytes and is excreted in the meninges when they are irritated or defective. Since lead is carried in the blood as the phosphate in colloidal solution, this theory of the transportation of lead is incorrect; but Camus' experimental observations are exceedingly important because they demonstrate the relation between the mental manifestations of lead poisoning and the pathological physiology of the meninges. Although there is general agreement that the choroid plexus ordinarily keeps toxins out of the cerebro-spinal fluid, certain pathological variations make possible the "secretion" or passage of toxins. Ducrot and Gautrelet (103) demonstrated that methyl violet injected into the blood soon appears in the spinal fluid, and therefore concluded that this dye temporarily paralyses the choroid epithelium. In cases of jaundice they observed that after methyl violet injections bile passes very easily into the spinal fluid. Also Mestrezat (302) found that in cases of meningitis the choroid plexus, which is ordinarily impermeable to sodium nitrate, permits this salt to pass quite readily. In fact, Levinson (259) demonstrated that when such substances as urea, chloride, and sugar are retained in the blood, their concentration in the spinal fluid increases proportionally. There is, therefore, sufficient evidence of variations in the permeability of the choroid epithelium during pathological conditions to suggest the possibility that such a mechanism may operate to produce lead encephalopathy.

Because patients suffering from encephalopathy are very violent, it is difficult to examine them completely, especially to perform lumbar punctures. Mosny and Malloizel (315) (316) have, however, been able to make routine lumbar taps and have found that the fluid is always under increased pressure and contains an increased number of lymphocytes (the count averaging 100 per cubic millimeter). These evidences of meningeal irritation during encephalopathy have also

been found by Norton (332), Boveri (50), and Troisier (466) who, in addition, noted that the globulin of the fluid was increased. Mass (283) described a case, which he called serous meningitis complicating lead poisoning, in which the great increase in cerebro-spinal fluid produced a marked hydrocephalus. Mosny divides lead meningo-encephalopathy into four classes—acute, subacute, mild, and latent—and believes that he can distinguish between them by the spinal fluid cell count. In the last few years several cases of lead meningitis have been reported in small children most of whom ingested the lead by eating the paint on their beds, or from lead compounds on their mother's skin (462) (449) (451). In all of these the spinal fluid showed definite signs of meningeal irritation. As might be expected with such small quantities of fluid as can ordinarily be obtained, tests for lead were negative. It is therefore interesting to note that 0.08 mgm. of lead were recovered from 80 cc. of cerebro-spinal fluid taken from one of our patients with lead encephalopathy.

This discussion indicates that in so-called lead encephalopathy the meninges are primarily involved, or in other words that the disease is really a meningopathy. But it is important to remember that the subarachnoid space is continuous with the tissues of the brain and with the nerves through the perineural lymph spaces (51) and that not only lead but also any pathological products formed by the action of lead on the meninges may pass along this route. Therefore the brain may be involved through the passage of cerebro-spinal fluid from the sheaths about the vessels to the perineural spaces. Thus, some encephalopathy may occur, and there is a possibility that lead amblyopia may be explained by a similar mechanism (158). One of the most interesting phases of this whole problem is the question of how lead can pass from the blood into the cerebro-spinal fluid. Further investigation is necessary to determine what physiological and pathological conditions render the meninges susceptible to chemical toxins circulating in the blood.

Part IV. Clinical

XVII. General Clinical Description and Diagnosis

Lead poisoning is characterized by several clear-cut and typical signs and symptoms directly referable to the absorption and action

of lead salts. It is striking that there is an enormous variation in the immunity of different individuals to intoxication. No test is now known which demonstrates before exposure the degree of susceptibility, but by careful and frequent medical examinations of exposed individuals it is possible to recognize susceptibility before intoxication has really developed.

There are, however, several factors which tend to reduce immunity. Certain races seem to be predisposed to lead poisoning. That negroes are particularly susceptible has been stated by Edsall (106) and also pointed out to us by several physicians in white lead factories where negro labor is used. Sex and age also influence immunity. Children are said to be more easily poisoned than adults (337); and the prevalence of sterility and the frequent occurrence of abortion among women exposed to lead is evidence of their greater susceptibility (25). The numerous references to the rôle of the general bodily condition in determining individual susceptibility and immunity emphasizes the importance of this factor. In his book, Oliver (page 207) states that "poverty and general deprivation predispose to plumbism"; while Edsall reports that "any pre-existing disease that reduces the resistance, perhaps chronic renal trouble especially, increases the liability to attack." "Any sudden unusual exposure, a drinking bout or an infection such as influenza" may produce acute symptoms (421). And finally, previous attacks of lead poisoning facilitate the later development of symptoms, usually similar to those which appeared originally but sometimes of quite different type. For instance, both colic and wrist drop may recur several times or a new manifestation such as encephalopathy may develop (421).

Acute lead poisoning. Although lead poisoning is generally considered to be of two types—acute and chronic—there is much confusion about the exact line of demarkation between them, for the signs and symptoms characteristic of the strictly acute type also may appear during crises in the chronic form of the disease. The distinction should be recognized, however, which restricts the term acute lead poisoning to the intoxication resulting from sudden absorption of relatively large quantities of lead or to the disturbances which are rarely seen to follow brief exposure. The differentiation is most clearly demonstrated in experimental animals. Indeed, Straub

(447) states that in animals acute lead poisoning is a physiological experiment; chronic poisoning a disease. After the rapid absorption of large amounts of lead the characteristic acute signs and symptoms are vomiting; gastro-intestinal irritation manifested by pain, diarrhea, and complete loss of appetite; general weakness; and a marked dehydration which may result in collapse or death. In some cases destruction of large numbers of red blood cells in the peripheral circulation results in acute anemia and even occasionally in true hemoglobinuria. Ockerblad (334) has recently reported a case in which the ingestion of 1.2 grams of lead acetate was followed by the appearance of a lead line around the teeth, stippling of red cells, marked hematuria, epigastric pain, and general malaise. In the literature are to be found descriptions of mild nervous disturbances or even of convulsions and coma following sudden absorption of lead. Stewart (441) states that five patients who had eaten bread colored with lead chromate died with convulsions; and Orfila (344) observed several cases of acute poisoning characterized by severe colic and coma. The signs and symptoms of acute plumbism in very susceptible individuals who have been exposed to lead for brief periods are those more commonly associated with the chronic form of the disease. The acute type may therefore be recognized, but lead poisoning is usually chronic as it is most frequently caused by gradual accumulation and storage of lead in the body. The sudden development of acute disturbances, however, may be similar in both types of the disease.

Chronic lead poisoning. To define early or mild chronic lead poisoning accurately is extremely difficult and necessitates clear differentiation between absorption, damage to body tissues, and intoxication. Evidence of absorption is the presence of lead in the excreta and the appearance of a lead line in the gums. While neither the presence of lead in the urine and feces, nor the discoloration of the gums demonstrates in itself that there has been any injury to the organism, both signify that lead has been absorbed.

Damage to body tissues may become manifest in many different ways. Lead may cause the early development of arteriosclerosis, or chronic nephritis as well as other degenerative changes which bear no

apparent relation to lead poisoning. This renders diagnosis very difficult. The rate of incidence of these pathological conditions is not clear, for information has been based chiefly upon poor industrial statistics.

The more severe types of intoxication by lead are very easy to recognize; but the mild manifestations are so protean in character and develop so irregularly that differentiation between absorption and true intoxication is often nearly impossible. Consequently, diagnosis must depend chiefly upon the number and intensity of the signs and symptoms.

For certain diagnosis of early chronic poisoning, it is usually essential that at least one typical sign of *absorption* be present, and also two distinct signs of a *generalized intoxication* by lead. There are four signs which most frequently appear during the early stage of the disease: (*a*) the ashen color of the skin, (*b*) the lead line on the gums, (*c*) stippling of the red blood cells, and (*d*) a mild secondary anemia or such other evidence of rapid peripheral destruction of blood as an increased quantity of blood pigment in the plasma or of hematoporphyrin in the urine.

Pallor of skin. Probably the most constant of the early signs is the ashen lead color of the face and pallor of the lips. Examination of the blood does not confirm the suggestion that this is caused by severe anemia alone, and Koelsch (238) believes that it is partly due to the action of lead on the skin capillaries. The exact cause, however, is not really known. Schmidt (400) agrees with Teleky (456) that this greyish pallor is very characteristic of lead poisoning, but thinks that intoxication may occur without it: a point of view which our experience confirms.

Lead line. As previously noted the so-called lead or Burtonian line was first described by Grisolle in 1835 (178), by Burton in 1839 (59) and mentioned at about the same time by Tanquerel (453). It consists of fine granules of pigment situated within the tissue of the gums about a millimeter from the border of the teeth. By insertion of a piece of paper under the gum, its location within the tissues and its punctate character can be readily demonstrated. Because the discoloration is sometimes very faint, the line may often be found only

by careful examination of the margin of the gums with a hand lens after all pus and detritus have been wiped away. Typically it appears around infected or pyorrheic teeth, but occasionally may be found in the buccal surfaces of the mucous membrane, i.e., on the inside of the lips or cheeks near decaying teeth; and frequently at autopsy in the mucosa of the colon and lower portion of the small intestine. It is stated that similar pigmentation may be caused by copper, bismuth, and silver, but that its localization is quite different.

Although the line may appear when exposure to lead has been very brief, it usually persists for several months after exposure has ceased; indeed, Teleky (456) reports that he has seen it eleven months after the cessation of exposure, and in one of our cases the coloration was evident for seven months when absorption of lead was prevented. In spite of the fact that the pigment lies actually within the tissues and cannot be rubbed off, careful cleansing of the mouth and teeth eliminates the line in large measure. We have never observed a recurrence of the line after it disappeared during the course of treatment, but in one patient suffering from acute plumbism, it did develop under the regular hospital regime without medication. Fagge (124), however, reports that in three cases of chronic plumbism, the administration of potassium iodide was followed by the appearance of the lead line.

The clinical significance of this sign should be properly recognized. It is an indication of absorption only, and not of intoxication, but may almost always be found during even slight poisoning. Its intensity and size provide a rough index of the duration and severity of exposure to lead, though it must be remembered that pyorrhea and dirty teeth markedly increase the rate of its development.

Stippling of the red blood cells. Another sign to which much importance has been attached is the appearance of stippling in the erythrocytes. Our experience as well as that of some other writers, including Schoenfeld (403), is that stippling is the most reliable of the early, rather indefinite signs and symptoms of lead intoxication. Although in many cases this basophilic granulation of the red cells is clear evidence of the presence of lead within the body, certain factors must be considered before assigning diagnostic value to the

stippling. Even normal blood contains a very few stippled red cells; while during some chronic diseases, such as pernicious anemia and the leukemias (176), and also in certain diseases of infancy including pneumonia (58), they are very frequently observed (see section XI). Consequently before stippling can be considered a sign of plumbism, the possibility of other disturbances must be ruled out, and the proportion of stippled cells must be large enough to show that the blood is really abnormal. Schmidt (400) has suggested that at least 100 cells in every million must be stippled before any diagnostic value can be assigned to the change. Our repeated daily examinations of the blood during plumbism confirm the observation of Meyer and Speroni (303) that the granulation varies markedly—it may be very intense one day and practically absent the next. It is therefore important to base diagnosis on more than one observation and to prepare slides with great care. (For methods see page 131.)

Although experience has proved that stippling is one of the most valuable of the early signs of lead intoxication, its exact significance is not known. Teleky (456) states that marked granulation is an index of the rapid absorption of lead, but the observation of marked stippling in one of our cases nine months after exposure contradicts his view and suggests that probably the red cells become stippled when lead is circulating through the organism and can affect both blood cells and body tissues. Therefore, whenever it appears in large numbers of cells, further exposure to lead should be prevented immediately. Both Teleky and Schoenfeld (457) report that stippling is more intense in early than in late severe plumbism; and several industrial writers confirm their belief by stating that it cannot be found in the blood of men who have been exposed to lead for long periods.

Anemia. Associated with stippling in the blood as one of the very early signs of the disease, is a constant mild secondary anemia. The mechanism by which this is produced has already been fully described (page 133). The reduction in the number of red cells is not extremely great, and it is rare to find less than three million per cubic millimeter. The microscopic appearance of the blood is that of typical secondary anemia except that some of the red cells contain

basophilic stippling. Evidence of regeneration of blood cells is manifested in early cases of intoxication by the increased number of reticulated cells. As the marked peripheral blood destruction increases, the amount of blood pigments in the serum dilution test is high and the quantity of pigments in the bile is often increased. The increased resistance of red blood cells to hypotonic salt solutions has been described by Malassez (275) and von Liebermann (266), and their observation has been confirmed by Orbán (343), and in this laboratory (15); but this is not usually sufficiently striking to be of much clinical aid in differential diagnosis.

Lead is said to produce a relative lymphocytosis, so that though the total number of white blood cells is not increased, the percentage of lymphocytes in the blood is raised (497) (422). Our differential counts, however, have not demonstrated this strikingly, for the average differential count has demonstrated about 28 per cent mononuclear cells.

Jaundice. The face and sclera suggest by their slight yellow tint that the intoxication has involved the liver (362). But the coloration is probaby due to an increase in the quantity of blood pigment in the plasma because of rapid destruction of red blood cells. Evidence of this is found in observations by Dr. Chester Jones (229) of increased pigments in the plasma and bile. It is true that Potain insisted that the liver diminished in size during lead colic, but other observers have explained the diminished liver dulness by dilatation of the intestines. Tanquerel (453) recognized two types of jaundice during lead poisoning. Before 1840 he made a differentiation between the two types by the statement that one was due to "extravasation of bile from its usual reservoir," and the other to "a direct change of the blood by lead." He saw only 51 cases of true jaundice in 1217 cases of lead colic.

Hematoporphyrin. In Germany many writers have considered the presence of hematoporphyrin in the urine an evidence of lead poisoning to which as much importance can be assigned as to stippling of the blood cells. That it does appear in the urine has been fairly well ascertained (456), but its true significance seems to be a matter of debate.

Gerbis (155) recognizes it as an important sign but believes that it cannot be used as certain evidence, for from 195 cases of lead poisoning he obtained the following data:

DEGREE OF STIPPLING OF BLOOD	NUMBER OF CASES	
	Showing hematoporphyrin	Not showing hematoporphyrin
Slight	5	13
Marked	12	4
Very marked	6	5

He does, nevertheless, consider that hematoporphyrin appears more constantly in the urine than does stippling in the blood, and gives data demonstrating that neither of these signs occurs in all cases. In six patients he could find neither stippling nor hematoporphyrin in spite of other good clinical evidence of lead poisoning. Curschman, quoted by Teleky (456), has also obtained data regarding its occurrence. He found that it was present in the urine of 30 per cent of a group of subjectively normal individuals who had been exposed to lead. In 40 per cent of these positive cases it finally disappeared, in 10 per cent it was still present when observations were stopped, and in the other 50 per cent definite symptoms of lead poisoning developed. Unlike Gerbis, Teleky, and Schmidt, however, Curschman is reported to believe that as much reliance cannot be placed upon hematoporphyrin in the urine as upon stippling of the red blood cells. And Schmidt (400) states that hematoporphyrin "is found in other diseases, as in rheumatism, heart failure with stasis, etc.," while Schoenfeld and Koelsch (238) have not been able to demonstrate that it appears constantly in the urine during plumbism.

Excretion of lead. The appearance of lead in the excreta is considered by many an early indication of lead poisoning. Murgia (323), for instance, states that it is the earliest and most constant sign. Too much weight should not be placed upon the presence of lead in the feces during exposure, for in analyzing these there is no means of distinguishing between lead which has been ingested and immediately excreted, and lead which has been actually absorbed. In the urine, on the other hand, lead is present only after absorption into the circulation. During exposure, lead is not always excreted,

for all that enters the organism may be retained in the tissues. Consequently, absence of lead in the excreta does not eliminate the possibility of exposure, and the presence of lead does not necessarily signify intoxication. The proportion of lead excreted by the two routes varies greatly. The quantity in the urine remains quite constant (see figs. 20, 21 and 22) even during medication; while that in the feces may become very large.

After exposure to lead has ceased, elimination in the excreta, especially in the feces, may continue for a very long time. We have found lead in the feces of a workman three years after he left his hazardous trade, and Oliver (337) has found it in the urine eleven years after removal from the hazard. Even when the excreta contain lead for such long periods, there is no justification for assuming that lead has produced deleterious effects. These, of course, should be looked for, but lead may and often does enter and leave the body without causing intoxication, and may at one time or another be excreted by any one exposed to lead.

Other signs. The other signs which commonly appear in the early stages of lead poisoning are not as constant as those already mentioned. A fine tremor may frequently be seen in early as well as in advanced cases. Although it is often not very striking, it is located in the muscles of the hand, the tongue, or face particularly around the eyes, and the eyelid, and is sometimes manifested as fibrillary twitching. It is easily confused with the tremors found in many other diseases, and cannot, therefore, possess much diagnostic importance; but its frequent appearance deserves notice.

Another early sign of plumbism often mentioned by industrial physicians is high blood pressure. In our cases this could not be observed, and it hardly seems probable that hypertension can be of any great value in the diagnosis of early lead poisoning.

Teleky (458) considers a diminished power to extend the wrists a very common early sign of the disease.

Associated with these signs are many symptoms, such as headache, indefinite weakness and lassitude, lack of appetite accompanied by loss of weight, nausea, constipation, metallic taste in the mouth,

and halitosis. These signs and symptoms together are characteristic of the early stages of lead poisoning.

Toxic episodes. Workmen exposed to lead may frequently show the early signs for a long time without any acute developments. Then suddenly one of the toxic episodes (called "symptomes episodiques" by Vibert (477)), may be precipitated. These are colic, palsy, encephalopathy, optical disturbances, and so-called lead arthralgia. Why some people develop these after only a few weeks of exposure to lead, while others show nothing for many years and then suddenly develop colic or wrist drop, is difficult to understand. But immunity is always relative, and may be broken down by several factors which influence the general physical condition. Oliver (337) mentions undernourishment and poor diet, and Edsall (106) chronic diseases, particularly nephritis, as predisposing influences. Likewise, an acute infection, such as pneumonia or influenza, is very apt to produce acute symptoms, as Marvin Shie (421) and also Dr. Brockway of Brooklyn[3] observed during the influenza epidemic of 1918. Delearde and Du Bois (93) and others have also noted that lead colic usually follows a few days of alcoholic excess; and Shufflebotham (427) has seen acute symptoms develop in a lead worker after injury.

Once an acute symptom has appeared, attacks follow with increasing ease. Lead colic, for instance, tends to recur, and muscles which have been paralyzed or weakened usually suffer the first injury on re-exposure. But succeeding attacks are not necessarily similar in character, and the acute symptoms of a second attack may be entirely different from those of the first. Marvin Shie describes cases in which wrist drop followed re-exposure to lead although muscular weakness had not been a previous symptom. Even without a second absorption of lead from external sources, attacks of lead poisoning may recur.

Colic. The most common and dramatic of the acute manifestations of plumbism is colic. Tanquerel reports that of 1493 cases of lead poisoning, 1207 suffered from this symptom—ten times as many as from palsy, and seventeen times as many as from encephalopathy. Consequently, it is usually the first acute episode to develop.

To most observers the term colic implies the severe intestinal pain

[3] Personal communication.

which so very frequently follows absorption of lead, but this is only a restricted use of the word. Since any of the smooth muscles of the body may be affected by lead, colic may be of various types. At times spasms involve the muscles of the bladder and prevent urination. Tanquerel describes involvement of the muscles of the testicle, and also spasm of the uterine and vaginal muscles which produces pain similar to that of child-birth. In giving enemas, we have observed contraction of the rectal sphincter on insertion of the catheter. By far the most common manifestation, however, is that which involves the intestinal tract.

While suffering from colic, patients are cold, pale, and often drenched with perspiration. They commonly bend over and writhe about the bed in intense pain. Between attacks they are usually very restless, probably because of the sense of constant pressure in the abdomen which persists even between the intermittent spasms. The pain is of a tearing or griping nature and its intensity varies markedly. It occurs intermittently but with no apparent regularity —attacks are separated by intervals of only a few minutes or several hours. Although usually below the umbilicus, the discomfort shifts from one part of the abdomen to another, and patients often show its location characteristically by spreading both hands widely over the lower part of the abdomen. Sometimes local areas of hyperesthesia which migrate are present.

Acute attacks of colic are nearly invariably preceded by several days of obstinate constipation. During both this preliminary period and the attack itself, there is often a desire to defecate; but there is usually complete inability to pass anything except sometimes a small quantity of mucus. The vomiting, which occurs quite frequently with the onset of pain, may be of very brief duration or may persist until the other acute symptoms subside. The vomitus usually contains bile, is green and frothy in appearance, and small in amount. Excretion of urine is decreased.

The appearance of the abdomen during attacks of colic is quite characteristic. It is rarely distended, but is usually scaphoid in shape and held tensely and rigidly. Between attacks of pain the abdomen has a generalized doughy resistance and there is no true abdominal spasm.

In duration as well as time of onset, colic is very variable. Of 31 cases which Tanquerel des Planches observed in the hospital without treatment, one recovered on the fourth day, one on the seventh, and eleven between the eighth and twelfth. In fifteen, colic persisted until treatment was begun after more than twelve days in the hospital. Brouardel (55) states that colic usually yields to treatment within eight days, but that if neglected it may continue for from two to five or six weeks. Usually, however, in our experience treatment brings relief within two days.

Differential diagnosis. Differentiation between lead colic and the abdominal inflammations which require prompt surgical treatment is often very difficult in spite of several marked characteristics. The history of exposure as well as other evidence of lead poisoning help markedly in establishing diagnosis. During true lead colic, the temperature rarely rises more than one degree. The number of white cells in the blood remains nearly normal, and any increase is caused by mononuclear rather than by polymorphonuclear leucocytes. The pulse is often remarkably slow. There is also no real muscular spasm, though the abdominal wall may feel generally doughy and resistant. There is also the usual history of marked constipation.

Prognosis. In itself lead colic is not dangerous. In fact, Lewin is said by Brouardel (55) to have collected data from six thousand cases which show that in this number death resulted from colic only once. Recovery is always fairly prompt and complete though constipation tends to persist. As has already been mentioned, a single acute attack of lead poisoning renders an individual more susceptible to future attacks. Distributed through the literature are histories of cases in which, without further exposure, colic has recurred many years after the primary exposure had ceased. This usually occurred after severe illness or alcoholic debauch or after the administration of potassium iodide.

Lead palsy. Another striking but less frequent manifestation of lead poisoning is palsy. This was recognized by the ancients and as early as 1656 Stockhusen (444) wrote a good treatise localizing the lesion. Many others, particularly De Haen (90) in 1771, studied it well clinically, but Tanquerel des Planches (453) and Duchesne

(102) in the early part of the nineteenth century gave the most satisfactory clinical description.

Probably when these investigators lived, lead palsy was a far more common manifestation of plumbism than it is at present, because exposure to lead was much more severe than now. But in only 13 per cent of the cases of lead poisoning seen by Tanquerel was it observed.

Prodromal signs. Usually before the typical symptoms of this type of lead poisoning become evident, prodromal signs appear. Colic, for instance, or other acute manifestations may occur, or a general lassitude accompanied by painful cramps or tremor of the muscles which are later involved. Sometimes formication, numbness or a dull ache, and very rarely hyperesthesia, develop in the injured regions. "Even when sensibility is in the normal state, patients experience a sensation of fatigue and heaviness in the paralyzed parts, principally in the articulations connected with affected muscles" (453).

The manifestations of palsy are very varied in character and severity. Muscular weakness may be the only sign of injury or motion may be completely lost. The appearance of symptoms may be quite gradual, or very rapid as in the cases reported by Schlapp (399) and by Windscheid (495) when paralysis developed suddenly and without warning after trauma. Shie (421) states that this may also occur upon re-exposure to lead.

The onset of paralysis does not appear to be closely related to length of exposure. In 102 cases observed by Tanquerel in industry, palsy developed in 9 during the first month of work; in 14 during the second; in 36 within the first two years; in 32 after ten years of exposure; in 13 after twenty years; and in one after exposure had continued for fifty-two years. Thus neuro-muscular lesions may develop at almost any stage of plumbism.

Perhaps the most satisfactory description of the different types of lead palsy has been given by Déjérine-Klumpke (92) in her book published in 1889. By far the most common form is the well-known wrist drop or antibrachial paralysis which may be limited to the extensor muscles of the middle and ring fingers. In one case which we have observed, in spite of continued exposure, such paralysis did

not spread within a year. But usually more and more muscles become involved—the extensors of the index and little fingers first, and those of the thumb somewhat later. The abductors and adductors of the fingers and the interosseous muscles of the hand are rarely attacked in this type. After the fingers are paralyzed, the wrist becomes weak, because of involvement of the extensor muscles of the forearm. In this stage, paralysis affects all the muscles innervated by the radial nerve, except the long supinator and anconeus, and usually the abductor of the thumb, which is never involved until wrist-drop is very marked. As lateral movement is thus rendered difficult, the hand is held in a characteristic position, bent somewhat toward the outer side of the arm. Swelling, due to accumulation of fluid in the tendon sheaths, is common. The paralyzed muscles show marked atrophy.

Another type of lead paralysis, termed "brachial" by Remak (380), involves the deltoid, biceps, anterior brachial and long supinator muscles of the upper arm. Occasionally the pectoral muscles are affected, and sometimes a true scapulo-humeral paralysis may develop. It is very rare for this to occur without wrist drop; but even when associated with palsy of the forearm and hand, this type of paralysis is not a common manifestation of lead poisoning. Evidence that recovery is prompt and fairly complete may be found in the report of three cases by Déjérine-Klumpke (92) and also in a case which we have recently observed. Although at first the patient could move neither arm away from his body and was suffering from typical paralysis of both the upper and lower arm, the "brachial" palsy yielded rapidly to treatment after about four or five weeks. Improvement of the muscles of the forearm, on the other hand, did not come for more than seven months although treatment was continued steadily.

A third form of lead palsy in the arms is the Aran-Duchenne type in which there is progressive atrophy of the thenar and hypothenar eminences, the interosseus muscles, and the small muscles of the hand. This occurs alone or with a complication of wrist drop, and is said by Moebius (312) to be very common in the left hand of file cutters. In view of the theory that fatigue plays an important rôle in determining the location of lead paralysis this observation is

of great interest, for in their work file cutters not only use particularly the muscles mentioned above but place the greatest strain upon the left hand.

Although not a frequent occurrence, paralysis of the lower limbs does develop during lead poisoning. In 97 cases of palsy Tanquerel observed it 15 times; and Thomas (461) reports that it occurred in 4 of his 31 cases. Of these 19, only 5 patients suffered from paralysis of the lower limbs without involvement of other parts of the body. Although sometimes generalized, this type of paralysis commonly develops in regions analogous to those affected by wrist drop—in the lateral peroneal muscles, the common extensor of the toes, and the extensor of the large toe. The other muscles of the leg, however, retain their normal strength.

In addition to these characteristic manifestations of lead palsy, certain more generalized disturbances may be observed during chronic poisoning. Paralysis may spread from the leg or hand until by involving the diaphragm or larynx it causes death. There are other generalized cord lesions which have been attributed to the action of lead. Putnam (370) reports several cases, which were proved to be a manifestation of plumbism by the presence of lead in the urine, which looked like lateral sclerosis; and those of several other investigators bore a strong resemblance to diffuse anterior poliomyelitis (369). A few cases of generalized paralysis, amyotrophic lateral sclerosis, and spastic paraplegia have been reported (461) (419) (109) (28) (318) (270) (311) which seem probably to be caused by lead. Putnam mentions that in five cases of pseudo-tabes, with incoördination and ataxia, in which lead was excreted in the urine, prompt recovery followed treatment. Recently, Kraffczyk (241) has reported an interesting case of cerebellar ataxia caused by lead. The red blood cells of his patient were stippled, and anemia, nystagmus, and general weakness with marked ataxia were noticeable. The muscles most affected were those most fatigued. In most of the reported cases of generalized cord lesions, however, sufficient evidence of lead poisoning is lacking.

A theory explaining the localization of lead paralysis was first clearly developed by Edinger (105). Manouvrier (280) noted in 1874 that in a series of cases, palsy was most evident in the hand

most used, a fact which he explained by direct absorption through the skin. Two years later Remak (380) pointed out that the muscle groups functionally related are involved in characteristic order irrespective of their innervation. Ten years later Moebius (312) reported that in file cutters, palsy usually develops in the thenar and hypothenar eminences of the left hand—muscles which are greatly fatigued in their work; and by 1908 Edinger (105) had fully developed his theory of the appearance of palsy in those muscles which are most used. This was followed in the next year by additional evidence from Teleky (455). This whole theory is very important and is but little disputed at present. The experimental data upon which it is based have already been discussed.

There are certain features of lead paralysis which facilitate diagnosis and should always be kept in mind. It is almost never associated with fever, and this fact, as well as absence of stiffness in the neck, localization in the most used muscles, and the occurrence of some of the various other signs of lead poisoning, must sometimes be the basis of differentiation between lead palsy and poliomyelitis. Progressive muscular atrophy, amyotrophic lateral sclerosis, cervical rib, arsenic or alcoholic neuritis, and similar conditions must also be differentiated.

If further absorption of lead is prevented as soon as paralysis develops, complete recovery usually occurs, although it is often very slow. Teleky states that continued exposure retards or prevents improvement. Wrist drop which has persisted for very long periods may gradually disappear almost entirely. It is interesting that the muscles first affected by lead palsy are the last to recover.

Lead encephalopathy. In almost every case of plumbism there is a change in the mental attitude which may be so slight as to be overlooked entirely, or may consist in general sluggishness and dulness, with poor memory, inability to concentrate, and a tendency towards restlessness and irritability. Although in some cases encephalopathy may develop suddenly and without warning, these same symptoms in a slightly exaggerated form often precede the onset of severe disturbances. There are in addition, certain other prodromata described by Grisolle (179) and Tanquerel which deserve mention. Any signs or symptoms of lead poisoning, no matter of what charac-

ter, may appear before encephalopathy becomes manifest; and restless sleep, general nervousness, mental depression, persistent headache, vertigo, faintness, transient paralysis, tremor of the hands, and tingling sensations may be noticeable shortly before the onset of general convulsions.

Fortunately, as industrial conditions have improved with fuller knowledge of lead poisoning, the incidence of lead encephalopathy has progressively decreased until this severe manifestation of plumbism has become infrequent. Most of the ancient writers speak of the frequent occurrence of epileptiform convulsions, but no good clinical description of lead encephalopathy appeared until the time of Grisolle. He published an article in 1836 in which he reports his observations and divides lead encephalopathy into three groups—convulsive, comatose, and delirious. He studied 29 cases of true encephalopathy, and describes very fully ten of these "accidents cérébraux." As these observations were so complete, Tanquerel's description of 72 cases added very little to an understanding of the nature of this type of lead poisoning. In spite of the many investigations which have been made since that time (489) (460), the only outstanding new clinical contribution is the differentiation between syphilitic and lead encephalopathy.

Of the three forms in which this type of lead poisoning is manifested, Grisolle found that the convulsive or epileptiform is the most common and serious. It occurred in fifteen of his twenty-nine cases and proved fatal in eleven. Six times the first evidence of the trouble was a severe convulsion. The nature of these attacks is very much like that of an epileptic seizure with clonic contraction of the whole body, frothing at the mouth, incontinence of urine and feces, followed by coma. Stewart (442) describes an epidemic of encephalopathy which occurred in a family after eating buns colored with lead chromate (approximately $\frac{2}{3}$ grains in each bun). With much difficulty he was able to elicit a history of colic and pain, accompanied by the vomiting of greenish material, before the sudden onset of convulsions. In these cases, attacks which resembled epilepsy recurred frequently and proved fatal in four of the six children. The literature on this type of epileptic encephalopathy has been well summarized by Badie (23) and Thieme (460) who have also collected many case his-

tories of epileptic seizures of all degrees of severity. In Boston within the last two years, we have seen four cases, three of which died. The other two types of encephalopathy mentioned by Grisolle are much less frequent and are really not distinct. Delirium occurred in seven of his twenty-nine cases, and in three which we observed. Six of these ten cases died. In only five of Grisolle's cases did coma develop. Kiernan (235) also describes a melancholic type which, when chronic, is associated with progressive mental deterioration.

In animals exposed to lead either in the laboratory or in factories working with lead compounds, unmistakable signs of lead encephalopathy have often been noted. Grisolle mentions this; and it is well known now among workmen that cats living around lead factories are affected with palsy and have convulsions after being exposed for some time. Porak (361) reports that many of his guinea pigs died in convulsions or with palsy during experimental poisoning; and in five of our animals similar symptoms developed (four cats, one rabbit). As all of these animals manifested practically the same disturbances, the history of only one will be given here.

Cat 222. A suspension of lead carbonate was administered intratracheally to this animal three times in nineteen days. Four days after the last injection there was lack of coördination. When left alone the cat lay quite still with its head bent over toward the left paw. If made to stand, it would start to walk with very rapid steps and would rush toward the right until it fell or hit the wall. It never turned toward the left. The cat lay quietly on either side, showed no rigidity in legs or paws, and no paralysis or wrist drop. Although there was no nystagmus if the animal faced directly forwards, nystagmus was quite definite when the head was turned to either side, particularly toward the right. On the second day of the disease severe clonic contraction developed, with the head retracted and the whole body stiffened and twitching. Death occurred six days after the last injection of lead during one of these convulsions. Post mortem examination showed that, although some of the lead had been deposited about the trachea, most had entered the lungs and had been deposited in the upper portion of the left lower lobe. Macroscopically, the rest of the body was not remarkable. No typical stippling could be demonstrated in blood smears taken before and after death, nor in any of the bone marrow cells. The red count six days before death was normal. Lead was well distributed throughout most of the body but there was none in the brain and

cord. Microscopic examination of the cerebellum and cord made by Dr. Mella disclosed nothing remarkable except that in the cerebellum there was swelling, chromatolysis, and vacuolization of almost all the Purkinje cells. Many of the nuclei were swollen, others were extruded, and the cell bodies were fragmented. Only rarely were normal cells seen. This was observed with the cresyl-violet stain. Van Humann stain, and phosphotungstic acid stain showed nothing abnormal either in the cerebellum or in the spinal cord. The meninges appeared normal.

In three cats severely poisoned with lead, convulsions occurred during exercise in a treadmill, and as attacks never appeared in normal animals during similar exercise, or in these poisoned cats at other times, fatigue must have been the immediate cause.

It is interesting that lead is not usually found in the spinal fluid. Thus, Strong (449) reports that he was unsuccessful in an attempt to find it in the fluid obtained from an infant who died of lead meningitis, and Kraffczyk (241) found none in material taken from a patient with chronic ataxic lead poisoning. Two specimens were examined in this laboratory. One was taken from a man who had mild plumbism but died two years later from general paresis, and, although 50 cc. of his spinal fluid was examined, no lead was found. The other was from a patient with marked encephalopathy, and microchemical quantities of lead were demonstrated in 80 cc.

As yet no satisfactory explanation for lead encephalopathy has been found.

Traube (464) suggested that edema of the brain might be the cause of symptoms, Rosenstein (390) mentions spasm of cerebral vessels and consequent anemia as a possible mechanism, and Westphal (489) believed that lead acts directly on the tissue of the brain, or establishes a condition of uremia upon which the symptoms depend. But all of these suggestions are based purely upon theory and no data have been obtained from which a satisfactory explanation may be derived.

On account of its similarity to other types of disease, differential diagnosis of lead encephalopathy is very difficult. It may simulate tumors and syphilis of the central nervous system, cerebral hemorrhage, epilepsy, chronic alcoholism, uremia, eclampsia, hysteria, and even neurasthenic disturbances; and great care must therefore

be employed in differentiation. By the appearance of certain signs, however, it is usually possible to distinguish the disturbances occasioned by lead. The lead line, stippling of the red blood cells, anemia, lead in the excreta, or colic may frequently be observed. On lumbar puncture, an increase in the pressure of the spinal fluid with an increase in the number of cells, especially of lymphocytes, is characteristic of lead encephalopathy; while the Wassermann test is negative.

Although statistics show that a large percentage of patients with lead encephalopathy die, it is dangerous to give actual figures, for in different reports the mortality rate varies from 25 to 75 per cent. If patients survive, their mentality sometimes remains distinctly changed. They are apt to have melancholia and delusions, or a poor memory and mental sluggishness, and, occasionally, epileptiform convulsions.

Optic lesions due to lead. That lead causes neuritis of the eye and palsy of the eye muscles has long been known. Tanquerel found but twelve eye lesions in his series and thought this complication very unusual, a point of view generally held, as for instance by de Schweinitz (410). And yet certain authors, such as Lockhardt Gibson (157) (158) of Australia, who in fifteen years saw 54 cases of ocular neuritis due to lead, insist that the effects of lead on the eye are not very rare. Of the various recent publications on the subject the articles by Lewin (261) in 1904 and Lockhardt Gibson are perhaps the most interesting. The lesions of the eye take various forms. Palsies and cramps of the various individual eye muscles, as well as blindness due to optic atrophy, and inflammation of the retina with the subsequent development of atrophy also occur. Hemianopsia occasionally develops. Lewin says also that corneal opacities are sometimes due to lead.

The lesions of the optic nerve, however, are interesting because they are unique, inasmuch as they involve a nerve of special sense. Several explanations have been advanced for this, for instance, Weiser (485) thinks that the action of lead may be due to a change in the blood vessels in the retina which causes hemorrhagic retinitis. Lockhardt Gibson, however, claims that nearly all the cases of toxic amblyopia due to lead have been associated with signs of encephalopathy or

of increased pressure in the spinal cord, and that it is usual for the optic disc to swell as much as three diopters. This is possibly the reason that a poison which does not attack sensory nerves should affect the optic nerve. This view is not generally held, however, and primary optic atrophy due to lead is said to occur rarely.

Gout. That pain in the muscles and joints is not infrequently associated with lead intoxication was first pointed out by Tanquerel (453). He gives a very full and satisfactory description of the so-called lead arthralgia, which appears in the joints and muscles of the extremities—more severely in the flexors than the extensors, and in the legs than in the arms. This pain has been repeatedly mentioned by other workers, and recently Wright (497) has stated that "indefinite pain in the muscles . . . . . is common," and indeed that "pain in the joints, especially of the knees and elbows, is an important and usually early symptom" of lead poisoning. He also says that "Bursitis is not infrequent. Numerous cases of elbow pain in connection with lead poisoning have been studied. In a few instances the pain was closely localized to the external epicondyle of the humerus, but the majority showed tenderness on pressure over the head of the radius, the site of a bursa described by Dr. Robert Osgood."

Saturnine gout has also been described. Although Skagge (432) is said to have observed gout in lead workers in 1764, and Garrod (153) in 1857, pointed out that it was quite common among lead workers in England, gout has not been considered a usual complication of lead poisoning. Hubner (221) states that it is really not an uncommon symptom in older workmen and that it may even be seen in young men. His description is that of the typical gout which most often affects only the large toe but sometimes spreads to other joints. Brouardel (56) also states that he has seen six cases of gout in lead workers. It may, therefore, be possible that lead intoxication and a gouty diathesis are associated, although we have not observed it.

Late manifestations. Generalized arteriosclerosis is often seen in individuals who have long been exposed to lead, and chronic nephritis of arteriosclerotic origin may also be observed. That these result from the action of lead cannot be proved, but their incidence in lead

workers is high. The symptoms and signs do not differ from those usually seen in arteriosclerosis due to other causes.

Summary. This chapter briefly describes the principal clinical manifestations of lead intoxication and the acute toxic episodes which develop during the disease.

XVIII. STANDARDS OF DIAGNOSIS

Individual opinion differs widely about the data necessary for definite diagnosis of lead poisoning. Some physicians believe that diagnosis cannot be certain without the development of at least one of the toxic episodes, while others consider such very meagre evidence as constipation and slight stippling of the red blood cells sufficient. Our experience seems to indicate, however, that the intensity and number of the signs and symptoms, the interval between their appearance and the beginning of exposure, and evidence of any injury to body tissues which may be directly attributed to lead, are all factors which must be considered in the establishment of diagnosis.

Great variation in the appearance of these usual signs and symptoms is common. Indeed, in some individuals marked absorption may be indicated by a single sign—a heavy lead line, for instance—without any subjective symptoms of intoxication; while in others, marked symptoms obviously referable to lead may develop although the usual early signs are entirely absent. Quite rarely in very susceptible individuals it is said that such an isolated manifestation as colic may be the only indication of poisoning. But such cases as this are not common and, in general, diagnosis of *intoxication* by lead as distinguished from *absorption* must be based upon the appearance of both subjective symptoms and objective signs.

A good standard of diagnosis has been suggested by Newman, McConnell, Spencer, and Phillips (330) in their book entitled *Lead Poisoning in the Pottery Trades*. They lay great stress on the determination of known or suspected exposure to lead and on personal history and physical examination as means of eliminating conditions which might simulate symptoms of lead poisoning. The recognized signs and symptoms they divide into two groups: The first of major symptoms; the second of more generalized and less acute manifestations. In their opinion a diagnosis of lead poisoning cannot be made

unless at least two of the systems in Group A are affected and the numerous minor symptoms of true lead intoxication are present. If, however, only one sign included in Group A may be found, at least two more must be present from separate divisions of Group B to establish early diagnosis. When only signs from Group B appear, at least three from separate divisions are necessary before a tentative diagnosis of lead poisoning may be considered.

Group A:

A. General appearance:
   Marked pallor and profound anemia.
B. Digestive system:
   Colic.
   Obstinate constipation.
C. Muscular system:
   Muscular incoördination.
D. Nervous system:
   Peripheral motor paralysis of certain extensor muscles (wrist and ankle drop) and atrophy of most used set of muscles.
E. Vascular system:
   Blood: Basophilic degeneration with diminished hemoglobin.
F. Special organs and findings:
   Gums: Lead line.
   Stools and urine: Lead.
   Miscarriage: Repeated.
   Liebermann's test: Positive.

Group B:

A. General appearance:
   Pallor.
   Anemia.
   Emaciation.
   Drawn expression.
B. Digestive system:
   Loss of appetite or repugnance to food.
   Breakfast anorexia.
   Vomiting on eating solid food.
   Sweetish or metallic taste.
   Gastric disturbances.
   Constipation.

Pain in abdomen.
Parotitis.

C. Muscular system:
Loss of strength.
Malaise and tiring easily.

D. Nervous system:
Headache.
Insomnia.
Mental lethargy.
Tremor.
Dizziness.
Convulsions.
Mental affections.
Encephalopathic conditions.
Arteritis.

E. Vascular system:
Arteriosclerosis.
Hypertension.

F. Special organs and findings:
Eyes: Impairment of vision, including muscular incoördination.
Joints: Various pains.
Blood: Basophilic degeneration with diminished hemoglobin.

Of its sort this is a satisfactory standard. But it has the distinct disadvantage of assigning to a single sign or symptom positive or negative significance, whereas as a matter of fact there is a subtle gradation which makes the final decision a matter of judgment. For instance, there may be one stippled cell or many in a blood smear, and the lead line may be marked or evident only under a magnifying lens. In the last analysis, therefore, the early diagnosis of lead poisoning must depend upon keen judgment, and no fast rules can be established.

In order to avoid further exposure and to prevent severe intoxication it is essential that lead poisoning be at least suspected very early. Although the appearance of any one of the toxic manifestations, in an individual known to be exposed to lead, is sufficient evidence for this, definite diagnosis cannot be made without more decisive data. Thus, either a history of exposure to lead or the presence of lead in the excreta must be ascertained, and at least one of the characteristic

signs included in Group A must have developed during exposure and be associated with other distinct evidences of the disease which have also followed absorption, before diagnosis can be certain. Very little weight can be placed on the mere presence of such common persistent symptoms as constipation, anorexia, headache, insomnia, etc., unless they have markedly increased since exposure, for lead workers are no more immune to the other diseases which might also cause these symptoms than are individuals who are not exposed to lead. Therefore the development of distinct toxic symptoms after exposure is necessary for definite diagnosis.

Toxicity. It is difficult to determine even roughly the dose of lead which should be considered toxic, for the quantities eliminated without absorption from the gastro-intestinal or respiratory tracts cannot be measured, and individual susceptibility varies greatly. Teleky, however, is quoted in Oliver's book (page 37) as believing that ingestion of a little more than 1 mgm. of lead per day for several months causes plumbism and that a daily dose of 10 mgm. causes severe symptoms in a short time. Legge (252) thinks that 2 mgm. per day may cause chronic poisoning, while Sollmann (434) from a summary of the literature concludes that, in man, daily ingestion of 0.2 to 0.3 mgm. of lead per kilo will in time produce phenomena of lead poisoning. He thinks, however, that more minute doses might possibly interfere with nutrition and resistance.

XIX. THE TREATMENT OF LEAD POISONING

In the treatment of lead poisoning three definite problems are involved: Prevention, treatment of acute symptoms, and treatment of the disease as a whole. Much has been written on prevention in the last decade, chiefly by Alice Hamilton in America, Legge and Goadby and Sir Thomas Oliver in England, and by Teleky in Germany, with the result that the incidence of lead poisoning has decreased markedly. A full discussion of the means used to prevent plumbism does not belong in this monograph, but it may be of interest to summarize briefly the various problems encountered in industry. The following outline has, for the most part, been taken from Hutton's (222) excellent review.

Prevention. Exposure to lead may be prevented in two ways: By devising suitable appliances and processes for manufacture; and by medical supervision of workmen. Since medical supervision can accomplish little when exposure is very severe, the first step is always to ascertain that every precaution has been taken to render factory conditions safe. The very rapid absorption of lead from the respiratory tract points to dust as the most dangerous source of lead. Consequently, every effort ought to be made to substitute wet processes for those in which dry and dusty chemicals are used; to store and transport lead in air-tight receptacles; to equip machines whenever necessary with suction drafts which draw dust from the workroom; and to cover pots of molten metal and place them under a suction hood so that the lead oxide, which forms as a scum on the surface, cannot blow about. Great emphasis must be placed on cleanliness, for if suction drafts become clogged, these precautions are useless. Dust should never be allowed to accumulate. Mechanical processes ought always to be substituted for manipulations by hand, if possible; and molten lead should not be heated above 700°C. on account of vaporization. White lead ought to be prepared only by moist processes, and as Klein (236) has demonstrated paint should be rubbed down only with abrasives, such as waxed sandpaper, which can be used on moistened surfaces.

Whenever mechanical devices fail to eliminate dust, work must be so arranged that as few men as possible are exposed, and careful instruction be given as to dangers involved.

Certain measures of hygiene are particularly important. Workmen should wear clean clothes while at work, and always keep them quite separate from those worn at other times. This requires two lockers, for if dusty clothes are hung at night where street clothes hang during the day, contamination can hardly be prevented. Smoking or chewing tobacco, if the hands are at all dirty, involves the danger of ingestion of lead; and as food may also be contaminated in the same way, provision must be made for washing thoroughly, and for eating in rooms completely removed from the lead hazard. Before dressing to leave the factory, workmen must be required to wash off all dust with great care.

Respirators have proved an unsatisfactory means of protection

because they either make breathing difficult or fail to collect all the dust. They usually fit so poorly that considerable dust enters between the edges of the mask and the cheeks. Consequently, they merely give a sense of security without providing adequate protection. While they may not be entirely discarded, it must always be remembered that the use of respirators does not remove responsibility for the elimination of dust in the workrooms. This is the duty of every factory utilizing lead or its salts.

Medical supervision should be thorough. Physical examinations determine the fitness of applicants for work, and any individuals who have had repeated attacks of lead colic or other form of lead intoxication, who show a marked degree of anemia, or evidence of nephritis, or high blood pressure ought to be rejected (337). Preferably only men should be employed, and only those who are in good physical condition and who are not mentally defective. During exposure to lead, employees must be carefully watched and examined every week or two in order to detect very early the development of anemia, pallor, lead line, or other symptoms of active lead intoxication. The physician should be given authority to order the transfer of suspicious cases from hazardous positions, or to request a period of rest. Upon re-exposure great emphasis must be placed on the necessary precautions, and the factory physician should be rigorous in his supervision.

The treatment of lead poisoning. Before the dangers of exposure to lead were understood, and when lead salts were used for medication and to sweeten and preserve wine, the only forms of lead intoxication recognized were the "symptomes épisodiques." According to the older physicians these required very drastic treatment, and many so-called "specifics" came to be employed. As colic is the most common manifestation of poisoning and responds most readily to treatment, more different treatments have been devised for it than for the other symptoms.

The oldest and probably the most renowned is that employed by the Italian monks who took charge of the Hôpital de Charité established in Paris in 1602 by Marie de Medicis. Their "specific," called "macaroni," consisted of one part of glass of antimony and two parts of sugar very carefully powdered and mixed. This was given in doses of one scruple for

three or four successive days. As time went on, however, treatment became more and more complicated until it consisted of the administration of violent purgatives, sedatives, and sudorifics. "C'était une méthode antique et spécifique, de laquelle il ne fallait pas même demander les raisons (453)," or as Dana translates the French, "It was a violent specific, but respectable from its antiquity." In 1836 Tanquerel (453) found that croton oil was just as effective as the more powerful traditional medication, and in treating his patients he usually employed this purgative. Although Moseley (314) and later Gendrin (154) advocated the use of sulphuric acid lemonades as very effective, Tanquerel did not consider their action particularly successful in relieving colic. Frequent reference is also made in the literature to the use of nux vomica, opium, antiphlogistics, and leeches, as well as to cupping and bleeding as suitable treatment for lead colic.

The first mention of a course of treatment to aid excretion of the store of lead within the body was made by Melsens (297) in 1840. He performed experiments on animals which demonstrated that potassium iodide when used with purgatives is a very effective form of medication; and concluded that it produces in the body lead compounds which are soluble and can be readily excreted. When administered to animals simultaneously with lead, he noticed that it prevented intoxication; in previously "leaded" animals, however, large doses caused fatal poisoning. Although Melsens obtained excellent results in treating his patients with potassium iodide, he did not analyze the excreta to determine the exact effect of this medication, and it was not until 1853 that Parkes (352) found lead in urine after administration of potassium iodide when it had not been excreted by this route before treatment. His observation was verified by Annuschat (10) in work on a dog with lead poisoning; and also by Pouchet (366) who noted that the rate of excretion is raised for only a few days, and falls promptly to the normal level. In 1893 Dixon Mann (279) carried out very careful investigations of the effect of potassium iodide which led him to conclude that although this drug apparently makes patients feel more comfortable, it does not increase excretion of lead in urine.

Thus, data concerning the efficacy of potassium iodide in the treatment of plumbism have been quite contradictory and further experiments were necessary to determine just how it produced its beneficial effects.

We have therefore analyzed both urine and feces repeatedly in this laboratory in an effort to throw some light on the problem. Our detailed data (see section IX) show that potassium iodide defi-

nitely increases excretion of lead—on the average approximately doubles the quantity eliminated—and that this increase appears for the most part in the feces. This accounts for the results reported in the literature, for previous investigators examined the urine only. The increased excretion of lead justifies the use of this drug after the subsidence of acute symptoms. It is best administered three times a day in doses increasing from 3 to 5 grains to the limit of tolerance. During acute attacks of plumbism no such medication should, of course, be given because the development of symptoms implies the presence of toxic quantities of lead in the blood.

That potassium iodide may precipitate an acute attack of lead poisoning in apparently healthy individuals long after exposure to lead has ceased, is repeatedly stated in the literature. Probably many authors mention this merely as a warning, for few definite cases are cited, but Oliver (337) Fagge (124) and Lazell (250) report observations worthy of consideration. Of Oliver's two cases, one is that of a woman of seventy-two, who is said to have died of "lead poisoning" after taking 7 grains of potassium iodide. As the patient was suffering from acute symptoms of poisoning before treatment was begun, this case does not illustrate the point as well as Oliver's second case. In this, administration of potassium iodide was promptly followed by the appearance of a lead line and wrist drop, although there had been no previous evidence of intoxication. In 1876, Fagge (124) reported three cases in which a lead line developed under similar conditions. Lazell (250) also has described the appearance of double foot drop, lead line, and digestive disturbances with colic in a man who had had severe lead poisoning sixteen years previously without subsequent exposure. Three months before the onset of this palsy the patient had had lobar pneumonia, and potassium iodine had evidently been given to relieve persistent lung involvement. Probably this tendency to promote disturbances is not restricted to potassium iodide alone, and any other treatment which liberates lead from the deposits in the body might precipitate acute symptoms by allowing too much lead to enter the blood stream (section IX).

Although so much importance has been ascribed to potassium iodide in the treatment of lead poisoning, it is not the most effective agent

for increasing excretion of lead from the body. References to the use of acid or alkalies are very few.

Gendrin (154), early in the nineteenth century, attempted to render lead salts inactive in the body by transforming them to insoluble sulphate with sulphuric acid. Sabrazès (393) mentions that acute symptoms developed in a case of late chronic plumbism after inordinate drinking of "citronade"; and Brouardel (56) states that sour salads will cause colic to recur. But the true significance of these observations on organic acids cannot be ascribed to the production of an acidosis.

The experiments reported in section IX indicate that acids and, to a less degree, alkalies are distinctly effective in increasing the elimination of lead. This is apparently due to two factors: a negative calcium balance, and a distortion of the normal acid-base equilibrium of the tissues. Marvin Shie (422) has already suggested as a result of his clinical observations that metabolic disturbances probably play a noteworthy rôle in the liberation of lead.

A new treatment for lead poisoning which has proved satisfactory in our cases is based upon our observations of the excretion of lead in cats and man. The foundation for this is the fact that the quantity of lead excreted may be varied by distorting the calcium metabolism, and that a positive calcium balance favors storage of lead, while a negative balance tends to increase the rate of excretion. The association of active symptoms with a marked distribution of lead throughout the body—in other words, an active lead stream—indicates that when symptoms of lead intoxication are evident it is wise to immobilize lead by impeding its liberation from the bones. Therefore, until an acute attack has disappeared the diet should contain much calcium in the form of milk and calcium lactate. As, in our experience, the normal minimal excretion of calcium per day, when the diet is deficient in calcium, appears to be 0.235 gram, and as a quart of milk contains approximately 1.1 gram, one quart of milk and 2 grams of calcium lactate should adequately fulfill the requirements. Under this treatment alone colic rapidly subsides, but it is advisable to give atropine and cathartics in addition.

We have not determined the actual effect of these various medica-

tions upon the duration of colic. To do this satisfactorily would require a very large number of observations, and there are in the literature practically no reports of the duration of untreated colic except the 31 cases of Tanquerel (453). In these, the average duration without relief was more than ten days. Nearly all of our patients have been practically relieved within forty-eight hours, and only two had definite cramps for a period of eight days. We have not observed colic without treatment nor with catharsis alone, but certainly merely a high calcium intake and catharsis cause it to subside rapidly. In the three cases of encephalopathy in which this treatment was used, striking recovery was very prompt.

Not until acute symptoms have entirely subsided should "deleading" be attempted. This is accomplished by maintaining a negative calcium balance and changing the hydrogen ion concentration of the blood. (For the basis of this treatment, see discussion on page 96.) To do this, either acid should be administered with a diet which contains very little calcium, or alkalies should be given alone. During this course of treatment, the diet contrasts sharply with that advocated while definite symptoms are manifest. Milk, eggs, green vegetables, and many fruits are omitted entirely. In our experience the following diet has proved satisfactory:

Meat
Liver
Potato
Rice
Tomatoes } cooked without milk
Canned corn
Bananas
Apples (peeled)
Tea and coffee without milk
Butter fat (prepared by melting butter in hot water and skimming off the butter fat)
Bread (prepared without milk, such as salt-free nephritic bread or soda bicarbonate biscuits or crackers)
Sugar—salt—pepper

Under this regime, acids mobilize lead effectually. Phosphoric acid has proved the most useful, although it is rather unpleasant to

take. We have found that when mixed with water and sweetened it tastes somewhat like lemonade and may be taken through a straw without discomfort. If the dose proves very disagreeable, a little gin or whiskey makes it more palatable. By rinsing out the mouth with water or a solution of sodium bicarbonate, any excess acid is easily removed and injury to the teeth avoided. Doses of 20 cc. of the dilute acid should be given in a glass of water every hour about ten times daily for several weeks. If acidosis becomes too severe, loss of appetite, headache, and general malaise supply evidence that the limits of tolerance of the patient have been overstepped and that medication must be reduced.

Another less distasteful form of medication is the administration of ammonium chloride which is probably transformed into hydrochloric acid and urea in the body, and so produces acidosis (183). This can be readily taken in doses of 1 gram given in a glassful of water, ten and sometimes twelve times a day. Throughout this treatment, diet should contain little calcium. Although not easy to administer, this medication is far more effective than other more simple forms and in eight of our cases has increased the average excretion of lead three-fold. Ammonium chloride is now employed instead of phosphoric acid because it is less distasteful.

Sodium bicarbonate likewise is effective. If given in large doses (20 to 40 grams a day) it more than doubles the elimination of lead and therefore produces an effect approximately similar to that of potassium iodide. The suggested explanations for its action are given in the chapter on Excretion. It is chiefly valuable when nephritis prevents the use of acids or potassium iodide, but even it must be employed guardedly.

Our observations, particularly on animals, indicate that there is no use in attempting to continue treatment until elimination of lead is complete. As this would doubtless require several years, it is probably desirable to eliminate only the most easily mobilized lead. Our very prolonged observations suggest that after a certain point elimination of lead becomes progressively more difficult. When this stage is reached, it seems more practical to favor retention in the bones by maintenance of a positive calcium balance.

Of the other recognized methods of dealing with lead poisoning,

the only one which seems to yield noteworthy results is catharsis. Although this has been used successfully since the Middle Ages, the exact means by which it brings relief had not been determined, so far as we could ascertain, until experiments were performed in this laboratory to determine its effect. This treatment does not increase the rate of excretion of lead markedly; it merely improves the general condition of the patient by cleansing the bowels and preventing the constipation which is characteristic of lead poisoning. Thus it cannot be considered a "specific," but its use is quite justified by the beneficial results which it produces. Saline cathartics are apparently the most satisfactory. For continuous use, Carlsbad salts are best, but magnesium sulphate is effective and may be employed. Regulation of the bowels without drugs is, of course, a much better type of treatment than periodic purging.

The value of bipolar electric currents in the prevention and treatment of lead poisoning is disputed. Oliver (336) used this method on the theory that in passing through the body electric currents split the salts of lead into ionic constituents which migrate toward the different poles—the acid radicals to the positive, the basic to the negative electrode. Lead should consequently be deposited at the negative pole. In 1913 Oliver reported that such currents cause marked improvement of symptoms and that lead really could be found in the water of the electrodes. In most instances, when the urine had contained lead before electrical treatment, "less lead was found in the urine after the bath" (336). Since this work was published, several clinical reports have appeared which suggest that good psychical results follow the use of electricity in plumbism, but no further experiments have been performed which give support to the treatment. Goadby, Oxley, Schnitter, and Böttrich are all opposed to this form of medication. Goadby (167) found that after lead has been ground up with liver tissue, electric currents are unable to remove it; and that they are quite ineffectual when applied to "leaded" animals. Oxley (349) believed that the current must necessarily be too low to remove more than a negligible quantity of lead, and that a very large proportion would be carried by other ions. In a series of cases he could observe no improvement in the symptoms after electrical treatment, and could detect no lead in the water

about the electrodes. Analysis showed that lead was not present in the urine of a case of chronic plumbism either before or after use of the electric current. Experiments on two rabbits confirmed these clinical observations. In Schnitter's (401) experience with twelve patients, similar treatment produced no visible effect upon the lead line, the degree of anemia, the amount of stippling in the blood, or the general condition. Because the work of these three investigators seems so convincing, we have made no attempt to further establish the value of electrical treatment. This would necessitate analysis of the feces as well as of the urine and electrolyte solutions for any lead which might be excreted. Since lead exists in the body for the most part as insoluble phosphate in the bones or as un-ionized phosphate in the blood stream, the probability that it can be affected by electricity is small.

Quite recently Ockerblad (334) has reported that hyposulphite of soda (0.6 gram injected into the vein daily) is beneficial. His use of this drug in a case of acute lead poisoning was suggested by the work of Ravaut (378) who administered it in cases of arsphenamine dermatitis to produce an insoluble sulphite of arsenic. McBride and Dennie (286), who have employed it in the treatment of both arsenic and mercury poisoning, also point out that sodium hyposulphite may be useful in such other acute metal intoxications as plumbism. Their treatment is based upon the theory that when the metal encounters this salt in the body a sulphite is precipitated which is insoluble and therefore not dangerous to the organism. That the small quantities of ionized lead in the body fluids may be temporarily reduced in this way, seems quite possible, but only when considerable thiosulphate remains in the blood stream. Although this method of treatment does not prevent the entrance of additional lead into the blood or a re-transformation of the insoluble lead into a toxic salt, it may prove valuable in dealing with acute active lead poisoning and deserves further study.

Special treatment of toxic episodes. *Colic.* Treatment often brings rapid relief to the severe pain of typical lead colic, even when it consists only of such general measures as the application of local pressure, heat, and moisture on the abdomen. Enemas, although they are sometimes difficult to administer because of rectal spasm,

and ineffective because of rapid expulsion, at times prove helpful in stopping pain. If diagnosis is certain and all possibility of an acute surgical abdomen is ruled out, catharsis is the best treatment. In our experience, magnesium sulphate (given in doses of one ounce) has proved most satisfactory. If this is not effective, the dose may be repeated. Adequate doses of such drugs as atropine, nitroglycerine, or amyl nitrite should also be used, but resort should be had to opiates, morphine and the like, only under extreme conditions.

In five successive cases of colic, the intravenous use of calcium chloride (15 cc. of a 5% solution given very slowly) promptly relieved the pain,—but this observation requires corroboration. With our patients, benzyl benzoate has seemed ineffective. Until the acute manifestations have subsided, a light diet containing much milk and calcium lactate (2 grams daily) should be given to favor storage of lead in the bones; but as soon as the crisis has passed efforts should be made to favor elimination of lead from the body.

Palsy. During the development of palsy, treatment which favors storage of lead should be used, and elimination should not be stimulated until the neuro-muscular signs become stationary. Local treatment by galvanism, massage and splints is then desirable, and the use of strychnine is frequently said to be effective. This treatment should be continued for many months, inasmuch as improvement nearly always results in the end.

Encephalopathy. Although the usual treatment for mania or convulsions is of course necessary, the prompt storage of lead is obviously essential in the treatment of encephalopathy. But this manifestation is so rarely seen now, that the effectiveness of treatment has not yet been determined. Of the six positive cases we have seen, three died without such treatment. The three patients, who were given large quantities of milk and calcium lactate with a little sodium bicarbonate, recovered promptly. A seventh somewhat questionable case, that of a child who had lead in the excreta, also recovered when milk was administered in large quantities. McCord (289) has recently reported a case at the Cincinnati Academy of Medicine of prompt recovery from an acute lead mania following the administration of alkali. When the acute symptoms have subsided, measures to increase the elimination of lead seem advisable.

After any of these acute manifestations of plumbism, return to a lead hazard is distinctly undesirable.

Late manifestations. The treatment of arteriosclerosis and of the arteriosclerotic nephritis which develop after long exposure to lead is similar to that for the more usual forms of these manifestations. It sometimes appears desirable to increase the rate of excretion of lead even in these cases but this should be done only with caution, as patients with severe nephritis often cannot well tolerate inorganic salts, particularly potassium iodide and acids. It is necessary to administer these only with great care. Sodium bicarbonate is usually the safest drug to use.

Summary. The treatment of lead poisoning, therefore, resolves itself into treatment for the toxic episodes and mobilization of the stored lead. Various treatments favoring elimination of stored lead are discussed, among them several new methods. During severe intoxication it is apparently wiser to facilitate *storage* of lead by ingestion of ample calcium, rather than to set it free. After the acute toxic episodes have passed, *elimination* of lead may be accelerated by a low calcium intake, acids and their ammonium salts, sodium bicarbonate, and potassium iodide. Caution should be exercised in using these forms of treatment on patients with nephritis.

XX. THE PREVALENCE OF INDUSTRIAL LEAD POISONING IN THE UNITED STATES

ALICE HAMILTON

The literature of industrial lead poisoning in the United States dates probably from the publication by Samuel Dana of Lowell in 1850 of a translation of "A Treatise on Lead Diseases" from the French of Tanquerel des Planches. A second impetus to the study of this subject was given in 1883 by J. J. Putnam (370) of Boston, who by detecting lead in the urine showed how much more prevalent was this form of intoxication than had been supposed. The material both clinical and pathological, from American sources, however, remained very scanty till the beginning of this century. During the last ten years very valuable additions have been made to our knowledge not only of the action of lead on the human body but of the prevalence of

occupational plumbism in various trades, the period of exposure before poisoning occurs, the different forms which the intoxication assumes under different working conditions, and the dangers which must be guarded against if men and women are to be protected when engaged in lead work. The following is a very brief summary of what has been published in this field in the United States.

A word must be said first as to the sources of our information on the subject. There are many difficulties in the way of the searcher for accurate data as to the incidence of plumbism in any one occupation for we have in this country no system of sickness insurance, such as obtains in the older industrial countries and provides an invaluable body of statistics, nor is there any uniformity in our factory inspection records, each state having its own methods and standards. The United States Census Reports cover deaths only and so do the records of the Industrial Life Insurance Companies. It follows that though it is sometimes possible to obtain full and accurate records from individual plants it has never yet been possible to say how much lead poisoning has occurred in any given industry in any given period of time,[5] and the statements made in this paper are only approximately accurate, for they are based upon incomplete investigations.

In lead mining the United States is under a great disadvantage as compared with the older countries because while in European lead mines plumbism is almost unknown[6] because old mines are deep and yield only the very poorly soluble lead sulphide ore, the mines in our western states are still near enough the surface to yield oxidized ores, carbonate, sulphate and oxides which are far more toxic. Missouri mines are now down below the water line but in Utah, especially in those areas where the mines are dry and dusty there is an amazing amount of plumbism. Information secured by Arthur Murray (324) for the United States Bureau of Mines showed that in Utah alone there were 468 cases of plumbism in lead miners in 1919 and 1920, the rate being 6.7 per 100 employed in 1919 and 5.0 in 1920, while in the

[5] The most nearly complete examination in this field was that made by the United States Public Health Service, of lead poisoning in the pottery trade in New Jersey, Pennsylvania, Ohio, and West Virginia, which was published in 1921 as Bulletin No. 116.

[6] According to Oliver (Dangerous Trades, London, 1902) no lead miner has died of plumbism in England for many years, and according to Rambousek (374) it was practically unknown among the miners of galena ore (lead sulphid) in Bohemia. However, Biondi (374) asserted that he had seen lead poisoning among the galena miners of Sardinia.

shallow, dusty mines of Frisco the rate for 1920 was no less than 27.0 per 100 employed.

In smelting and refining lead there is a high rate of lead poisoning and this industry is very important in the United States and gives employment to a large force of men. No recent investigation has been made in this country and the only figures available are those collected by the Bureau of Labor Statistics in 1913 (471). During that year there were at least 1770 cases in 19 plants employing some 7500 men and among these were 41 encephalopathies, 35 cases of paralysis and 16 deaths.[7] The British rate for 1912 was only 1.8 per 100. Interviews with 167 smelter men, suffering from plumbism, showed that the majority had become intoxicated after only a short exposure. Eighteen had worked less than a month; 121 were exposed less than six months; 132 less than a year; and only 35 had worked more than a year, of whom 5 had worked more than five years.

That lead poisoning is caused chiefly by the presence of finely divided lead compounds in the air is abundantly shown in the smelting and refining industry. Thus, three smelters in which ore hearths (or Scotch Hearths), with their enormous flue and bag-house attachments, are used were employing 13.5 per cent of the total laborers in the trade but they had 22.4 per cent of the cases of plumbism, 54.3 per cent of the cases of palsy, 56.3 per cent of the fatal cases and 70.7 per cent of the encephalopathies. The greatest danger lies in the fumes of lead oxide and sulphate from the furnaces and next to that in the fine dust from the flues and bag-house. The two large smelters which have been recently investigated have installed many improvements and there is certainly less danger now in this branch of lead work than there was ten years ago, although it is probable that the refining end of the industry has undergone less improvement than the smelting because all over our country there are cheaply constructed and cheaply managed refineries, practically junk shops, where no effort is made to control dust or carry off fumes.

Zinc smelting may involve similar dangers if the blende contains much lead, as it does in Colorado. Missouri blende contains only 0.5 to 1 per cent, and New Jersey blende is almost lead-free.

[7] Of the 19 plants included in this study, only two had full medical records, and from four no information could be secured. Therefore, the number of cases which actually occurred in 1912 was greater than 1769, the number of records actually secured.

Metallic lead is used in a wide variety of industries where it is cast or molded into various shapes. Lead wire, sheet lead, pipe, machine parts, plumbers' goods, bullets and shrapnel, picture frames, coffin ornaments, grids for storage battery plates, car and can seals, stoppers for bottles and for basins, lead foil, printer's type, all are objects the making of which involves the handling of solid or molten metallic lead, and cases of industrial lead poisoning are on record in connection with all of these occupations. Other industries involving an extensive use of metallic lead are the making and use of solder, lead burning with an oxyhydrogen flame, and lead tempering of machine parts, piano wires, magnetos, etc. The plumbers' trade is less a lead industry than formerly, but repair work with "joint wiping" means exposure to lead. Among 560 cases of industrial plumbism collected by the Illinois Occupational Disease Commission, 19 were in plumbers.

The danger of plumbism in all these trades is comparatively slight and easily controlled. It is only necessary to carry off the fumes from lead pots, which is usually a simple matter, and to insist on strict cleanliness, no scattering of lead scrap and dust. But the very lack of evident and immediate danger in the use of metallic lead seems to induce a neglect of ordinary precautions and in many plants where soldering or re-melting used metal or casting is carried on, there may be more lead poisoning than in a very well managed white lead plant. Lead burners, who melt together two surfaces of lead with an oxyhydrogen flame, have probably the most dangerous work among the metallic lead occupations because of the great heat used. Next to that would come tempering metallic objects in a bath of lead, and later on brushing off the gray oxide coating which has formed.

Brass foundry work, especially pouring, is a not uncommon source of lead poisoning, for brass usually contains lead, sometimes more than 10 per cent, and both founders and polishers may suffer from typical plumbism.

The printers' trade is the most important of the metallic lead trades, and yet the exposure to lead is very slight. Typical lead poisoning has never been common in this industry,[8] but the trade is a notoriously

[8] In 1916 a group of 100 Chicago printers were examined by John D. Ellis, and a similar group of Boston printers by Walter W. Palmer. They found 18 men suffering from symptoms suggestive of chronic plumbism and with a history pointing to the same. This made a rate of 9 per hundred.

unhealthful one in every country (474). Recent statistics gathered by the International Typographical Union (New York Local) show that certain chronic diseases not clearly connected with lead are very prevalent among printers. There is an excess of early deaths from heart disease, chronic disease of the kidneys, apoplexy and paralysis. Thus, among white males in the registration area, only 18.6 per cent of the deaths from heart disease occurred in the age groups under forty-five years, but among the printers 26 per cent was the figure. The deaths from apoplexy and paralysis show an even greater contrast, only 9.1 per cent of the deaths from these causes occurring under forty-five years in the general group but 20.4 per cent among printers.

Tuberculosis is, however, the occupational disease of the printer and apparently it is connected with this exposure to lead, for in every country it has been found that when lead fumes and dust in the printing shops were controlled the tuberculosis rate always fell.

The statistics of the Metropolitan Life Insurance Company for 1917 show that printers have a higher tuberculosis rate and die from this disease at an earlier age than workers in non-metallic lead, such as painters. Tuberculosis is the cause of 34.1 per cent of the deaths of printers, of only 21.9 per cent of the deaths of painters and varnishers, and the former average only $33\frac{1}{2}$ years at death, the latter almost 40 years. A possible explanation for what is apparently the effect of tiny quantities of metallic lead dust inhaled over a long period of years, is to be found in the paper by Fine (137) to which the reader is referred.

Lead is roasted to form the oxides: Litharge, red lead, and orange mineral, for which there is a very extensive demand in industry. The danger comes from fumes from the furnaces, very much lessened in late years by the introduction of mechanical devices, and from dust in transporting the oxides, dumping, grinding, screening, and packing. The dust problem is much harder to solve than fume control. The newer methods of roasting require fewer men, but the number of oxide men nevertheless is decidedly on the increase because of the constantly growing demand for oxides to make storage batteries, enamels for sanitary ware, glaze for tiles and terra-cotta, glass and varnish, paint and rubber, to mention only the most important uses. Lead poisoning in oxide men is likely to be of rapid development and fairly severe (472).

The making of storage batteries involves the use of great quantities of lead oxides and this is not only one of the most extensive of the dangerous lead trades but the one that is increasing most rapidly. In Great Britain it is at present the greatest single source of plumbism. The rate in the five large plants in the United States in 1914 was a little less than 18 per 100, but this figure was below the truth, for almost no records could be obtained from the worst of these plants. The men engaged in casting the lead grids had a rate of only 1.7 per cent, but those employed in mixing the oxide paste had a rate of 40 per cent, and the pasters a rate of 19.4.

Enamels for bath tubs, sinks, and other plumbing goods also require a great deal of red lead, and here also the number of men exposed is continually growing for we make more new bath tubs every year. These enamels contained in 1911 from 1.5 to 20 per cent of soluble lead (soluble in human gastric juice). Lead renders the enamel more fusible and flexible. The dry enamel is scattered over the ware while it is red hot and there is no way of carrying off the lead laden dust so as to prevent contamination of the air, while the heat and exertion are too great to allow the enameller to wear a respirator. However, efforts to lessen the dust have been made of late and it is highly probable that plumbism is not so prevalent in this industry as it was in 1911 when 54 out of 148 men, who were examined, had unmistakable signs of chronic lead poisoning (469).

The manufacture of rubber, especially footwear, mechanical rubber, and automobile tires, uses now less lead relatively but more absolutely because so much more rubber is manufactured each year. The lead is usually litharge, sometimes sublimed lead sulphate, and it is added to crude rubber to accelerate vulcanization and to make the product heavier. Lately organic accelerators have been taking the place of lead and yet so great is the increase of rubber manufacture that there is more lead sold now to rubber factories than before. Only a small proportion of the employees come in contact with lead but unless great precautions against dust are adopted there may be a high rate of plumbism among the compounders and mixers. In one factory there were only 25 out of 1200 employees who handled lead but 11 cases of plumbism occurred in one year (473).

White lead is made by two methods—the old Dutch process and the

Carter. The former is slow and requires much hand work, the latter is dusty but quick and largely mechanical. This industry has undergone very radical improvement in the last ten years and almost all white lead works are now equipped with excellent mechanical devices to prevent dust, with ample washing facilities, a clean lunch room and washable working clothes, provided and laundered by the management. There is always a physician in charge of such plants. Cases of acute lead poisoning are fairly infrequent at present but every now and then a serious case of acute and rapidly developing lead poisoning does occur, showing that susceptible men run a risk in such work. Negroes are universally found to be more susceptible, especially to the cerebral forms of plumbism.

The painters' trade in every country has been brought under less complete control than any other lead trade, and it is the one that shows the least improvement in the rate of lead poisoning. For this reason the French are insisting on the abolition of lead paint and they have already closed their white lead factories and forbidden the use of lead paint on buildings. On the other hand, the British (338) (12) and German (255) (404) authorities on industrial hygiene lay stress on the toxic constituents other than lead which are present in almost all paint and also in paint removers, shellac and varnish. These are volatile fluids such as turpentine, benzene, petroleum distillates and wood alcohol, all of which are capable of producing more or less serious intoxication and, according to this view, are really responsible for much of the ill health of painters which is usually attributed to lead. (See abstract of controversy before the International Labor Bureau of the League of Nations, by E. L. Collis in the *Journal of Industrial Hygiene*, 1923, 5, 52.[9])

Nevertheless, great efforts have been made in Great Britain and in most Continental countries to minimize the dangers of lead paint by prohibiting dry rubbing down when lead paint is used, prohibiting the mixing of dry white lead by the painter, restricting the use of white lead paint in interior work, etc. Fortunately the American tendency

[9] The Draft Convention of the International Labour Office of the League of Nations which was formulated at the October 1921 session, and submitted to nations which are members of the League, prohibits the use of white lead or sublimed sulphate of lead in the interior painting of buildings.

to speed up the job leads more and more to the abandonment of dry rubbing down in interior decoration, except on very high class work, but it is still done in the painting of Ford automobile bodies and of practically all vehicle wheels.

The general impression obtains that less lead paint is used now than formerly, but interviews with white lead producers do not confirm this. There has certainly been an increased production of white lead in the United States since the War, and the painting trade is still the largest consumer, with the potteries coming next. A new danger to the painter has appeared recently in the introduction of the "spray gun" which has met with an enthusiastic reception and has spread rapidly. It is now used for painting all sorts of factory goods, not only small objects which may be placed in a cabinet with an exhaust, but automobiles, wagons, railway and trolley cars, agricultural machinery, furniture, and even the interiors of buildings. It is easy to see how this increases the danger of lead poisoning, for a spray of finely divided droplets, each carrying its tiny load of lead, is hard to control. The experiments of Sharpe (418), of Toronto, have shown that in such work the lead content of the air may go far beyond the limit of safety, and that the ordinary respirator lets a dangerous amount pass through. The Bureau of Mines has recently shown that a gas mask can be constructed which will hold back the lead, but it is certainly doubtful whether painters will consent to work in such a mask.

The painters' trade is still at the head of the list of occupations which give rise to plumbism, as is indicated by many recent reports in the United States (468) (470) (201). It must be remembered that an allied industry, that of the glaziers, is also attended with the use of white lead. This is a constituent of glaziers' putty, and recently four glaziers, working in a New York shop, were found to be excreting lead in the urine.

Pottery is the next most important of the white lead using industries and one that is increasing steadily in extent in the United States. Lead glaze is used on all so-called white ware (table and toilet ware), on smaller sanitary ware (not earthenware bath tubs), on art ware, yellow ware and Rockingham, and on glazed tiles used for interior decoration and occasionally on glazed roof tiles. White lead is the

form most used; next to that comes red lead. In some potteries the lead is "fritted," that is, it is added to the raw constituents of the glaze and fused with them and thus changed in part to the insoluble lead disilicate, a procedure which greatly lessens the danger, but which is unfortunately not often used in the United States, the lead being commonly added after fritting is over. The study made recently by the United States Public Health Service (Bulletin 116) shows a very high rate of plumbism among the men and women who glaze and decorate pottery and tiles. The physicians examined 1809 men and women potters and found a rate of at least 13.5 per hundred employed, while the rate in British potteries in 1913 was only 0.9 per hundred, one-fifteenth of the American rate.

The prevention of lead poisoning may be difficult of execution, but the principles which must guide it are very simple. In almost all cases of industrial plumbism and in all serious cases, it will be found that the workman has been poisoned by lead which entered his body with the inspired air. It is unnecessary to enlarge on this point, for the preceding chapters provide the explanation for a fact which has been observed repeatedly in practice since it was first announced by Tanquerel des Planches a hundred years ago. He said, "All the characteristic traits of the primary effects of plumbism may be quickly observed in workmen who are habitually in an atmosphere of lead dust and vapors. None of the primary effects are found among workmen who handle lead in a fixed state."

The practical experience of British and German factory inspectors bears out abundantly the assertion that the air must be kept free from lead dust and lead fumes (fume is a suspension of extremely fine particles of lead oxides and sulphate) if serious poisoning is to be prevented. Of secondary importance is the provision for personal cleanliness, but of course this should never be neglected.

Since the susceptibility to lead varies enormously in different individuals, and since it is impossible to detect this susceptibility at the time when the worker is employed, it is essential to have a medical examination of all lead workers at regular intervals. If any case of lead poisoning is discovered, the worker should at once be suspended from lead work and given other employment, preferably out of doors, till all symptoms of lead poisoning have disappeared.

XXI. ADDENDUM

Lead in liquor

In a few cases in which there had been no industrial exposure, lead poisoning has been caused by the consumption of alcoholic drinks containing lead. These beverages were illegally made—presumably with ordinary household appliances. Because lead was found in a few samples, various liquors known to be used commonly by saturnines have been systematically investigated. The results are grouped in the following table:

The occurrence of lead in liquor

	mgm. of Pb per liter of liquor
Distilled liquor	5.15
Distilled liquor	1.41
Distilled liquor	52.70
Distilled liquor	18.30
Distilled liquor	5.84
Distilled liquor	6.25
Distilled liquor	0.60
Wine	74.25
Wine	0.00
Wine	75.40
Wine	1.38
Wine	1.62
Wine	1.09
Wine	0.40
Wine	0.00
Wine	0.26
Wine	1.04
Wine	trace

It is interesting and significant that of eighteen samples of liquor analyzed, two only were negative. In the preparation of at least one kind of distilled liquor a lead worm had been used as a condenser. That wines should contain lead is not surprising as fruit acids (chiefly tartaric) are an excellent solvent for most lead compounds and can easily dissolve it from crocks glazed with a lead glaze, metal vessels containing solder, or from copper vessels tinned with an inferior tin-lead alloy. Consequently, contaminated liquors constitute a definite source for so-called "normal" lead.

The literature of plumbism contains many references to poisoning from liquors purposely or accidently contaminated with lead. Chevallier and Olivier (79) cite an instance of an epidemic of lead poisoning caused by the use of litharge in treating "green" wine. Campbell (62) and Allden (6) both report cases of lead poisoning occasioned by home made wine which had dissolved lead from the containers. Bidwell (37) reports contamination of liquor with lead and recently mention has been made (500) of several cases of lead poisoning in England caused by lead from the vitreous enamel coating of the cast iron tanks used in the manufacture of beer. Vaughan (475) also cites instances of lead poisoning after the consumption of "moonshine" whiskey which had evidently been contaminated by the stills.

The wide-spread use of containers glazed with lead compounds and the careless use of leaden or soldered containers in the preparation of home-made beverages is therefore a matter of concern from the view point of health.

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ETIOLOGY OF SCARLET FEVER

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Scarlet fever is in all probability a very old disease. The regions in which the malady originally arose are a matter of uncertainty. There are some who believe that the Plague at Athens was a malignant form of scarlet fever, an interesting assumption in view of the present relatively low case fatality and the ominous variability in severity of outbreaks in the past. Fairly accurate descriptive records of scarlet fever appear in the literature as early as the middle of the sixteenth century and recur with increasing frequency and definiteness up to the time of Sydenham. For many years the disease was confused with measles, erysipelas, diphtheria and certain septic processes. Sydenham, who first employed the name scarlet fever clearly differentiated it from measles by his careful description of the disease as it appeared in London from 1661 to 1675, and laid the foundation of an accurate knowledge of its special characters. In spite of this valuable contribution the existing confusion did not disappear, and many physicians still confounded it with diphtheria and certain septic anginas. With the increasing volume of medical literature and better facilities for the communication of ideas, scarlet fever became more and more clearly defined as a clinical entity. Confusion, however, with diphtheria frequently occurred, even, down to the times of accurate diagnosis by means of bacteriological methods. In fact even today the inability to differentiate scarlet fever from certain septic conditions of the throat, associated with erythematous rashes, continues to plague the mind of the diagnostician. In spite of these diagnostic difficulties clinical differentiation of scarlet fever from other exanthemata has been possible for a long enough period of time to determine clearly its contagious nature and to permit illuminating epidemiological and clinical studies.

The contagious element in scarlet fever is probably always derived

from a previous case. In most instances it is taken directly into the mouth or nasopharynx by the inhalation of air charged with minute droplets of saliva or mucous projected from the mouth or nose of the infected individual. Other important sources of contagion are the purulent discharges from infected paranasal sinuses, from suppurative inflammation of the middle ear and lymph glands, secondary conditions that constitute the most frequent and distressing complications of the disease. There is much evidence to support the view that the causative organism survives in the dry state in a virulent form for long periods of time. Contamination, therefore, of clothing or personal articles of any kind with infective matter may serve as a means of conveying scarlet fever. Formerly the belief was quite prevalent that flakes of skin given off during the period of desquamation were the most important vehicle of the contagion, and quarantine regulations were roughly founded on time periods corresponding with the duration of the desquamative stage. Current opinion holds that the contagious element is not present in the skin in the late stages of scarlet fever, a somewhat curious fact, inasmuch as the rash is the most distinctive clinical manifestation of the disease. The rôle of the healthy carrier in spreading scarlatina is undoubtedly of great importance but determined accurately as yet in only a very few instances because of the uncertainty concerning the etiological agent. That such types of carriers do exist there can be no doubt, and Bliss (1) has been able to trace a small epidemic of scarlet fever to such a source. Another interesting means of the wide dissemination of scarlet fever is an infected milk supply and numerous undoubted outbreaks have arisen from the consumption of contaminated milk. The clinical and epidemiological evidence, therefore, that has been collected indicates that the causative agent of scarlet fever is present in the throat secretions and the discharges from suppurative foci in patients throughout the illness, and for a considerable period of time during convalescence. It resists exposure to light, and in the dry state may retain its infectivity for many months. Healthy carriers and atypical attacks of the disease are not an infrequent occurrence. In all probability the udder of the cow may become infected with the specific virus, and the milk obtained from this animal may serve as a vehicle of infection.

Notwithstanding these excellent clinical and epidemiological studies which have ensured the easy recognition of typical attacks of the disease, and which have furnished the essential data for useful quarantine regulations, the causative organism of scarlet fever has remained unknown. Experimental studies have been publishd from time to time, suggesting that the infective agent belongs to one or other of the principal groups of microörganisms, such as the pathogenic bacteria, protozoa, and the so-called ultramicroscopic viruses. As a bacterial cause, Streptococcus hemolyticus has aroused much interest and stimulated more or less continuous investigation because of its constant association both with the uncomplicated and complicated forms of the disease. Certain observers have discovered inclusion bodies in leucocytes and in epidermal cells which they have thought indicative of a protozoan cause for scarlet fever. Finally, scientific opinion seized upon those mysterious living bodies commonly designated as filterable viruses as the most probable cause of the disease. This latter view has become most widely accepted and is the usual etiology assigned in text books in spite of the fact that no real evidence has ever been produced to show that any such microörganism exists either in the throat secretions, tissues or blood of an individual suffering from scarlatina.

Both Mallory (2) and Döhlé (3) have made the suggestion that scarlet fever may be due to a protozoan infection. In 1904 Mallory observed in four cases of scarlet fever certain bodies whose varying morphology strongly suggested that they might have been stages in the developmental cycle of a protozoan. They occurred in and between the epithelial cells of the epidermis and free in the superficial lymph vessels and spaces of the corium. They formed a series of bodies including definite rosettes, which closely resembled those seen in the asexual development of the malarial parasite. There were also certain coarsely reticulated forms which he thought might represent stages in sporogony. Mallory was of the opinion personally that these bodies were protozoa and bore an etiological relationship to scarlet fever, but he did not regard their significance as established. Confirmatory observations were subsequently made by Duval (4), Bernhardt (5) and v. Prowaçek (6). Similar bodies, however, were later found by Field (7) in other conditions and they

finally came to be looked upon as peculiar products of cell degeneration and not as living forms with a specific relationship to scarlet fever.

In 1912 Döhlé, on examining the blood smears from thirty cases of scarlet fever, found within the cytoplasm of the neutrophilic polynuclear leucocytes multiform inclusion bodies. These inclusions were present in a large percentage of all leucocytes and by special methods of staining revealed themselves as intermediate in intensity between nucleus and cytoplasm. In a later communication certain of these inclusions are designated as "Spirochaeta scarlatinae," and are assigned both diagnostic and prognostic importance. Although numerous observers confirmed Döhlé's observations on the presence of leucocytic inclusion bodies, further study revealed the fact that they are present in practically all febrile conditions, in chronic pyogenic infections without fever, in certain severe injuries, and occasionally in normal human beings. In all likelihood they result from nuclear degeneration not infrequently observed in septic states and have no specific bearing on the etiology of scarlatina. The evidence offered in favor of the protozoan origin of scarlet fever has never stood the test of close scrutiny.

The belief that scarlet fever is due to an unknown virus, probably of filtrable character, is based largely upon the results of attempts to communicate the disease experimentally to animals. A number of observers have reported scarlatina-like manifestations in monkeys inoculated with infective material from active human cases of the disease. Among these observations the most interesting are those of Levaditi, Landsteiner and Prasek (8), who, by the inoculation of anthropoid apes, seem to have produced what in all likelihood was true scarlatina. Exudate from the throats of individuals with scarlet fever was rubbed into the tonsils of apes and defibrinated blood injected subcutaneously, and in one instance material from a suppurating lymph gland. The animals, after an incubation period of about three days, are described as having a typical angina with characteristic exudate, enlargement of the follicles of the tongue, a generalized exanthem resembling that of scarlet fever, and in certain instances when the animals recovered desquamation of the skin. There was also present the characteristic lymphoid hyper-

plasia, and the histological lesions in the skin resembled those seen in scarlet fever. In all the animals presenting such a picture S. hemolyticus was present, either in the blood or in the local lesions in the throat. Levaditi, Landsteiner and Prasek, however, did not think that streptococcus was accountable for the manifestations, inasmuch as when pure cultures of this organism were obtained from the infected animals or from human beings and inoculated into fresh apes, the phenomenon described could not be reproduced. They do not state that the organism of scarlet fever is a filtrable virus, but simply say that it is of unknown characteristics. Cantacuzene (9) and Bernhardt (10) claim to have induced a similar series of phenomena by the inoculation of monkeys of a lower order with human material. Levaditi, Landsteiner and Prasek failed to produce in a large series of lower monkeys the disease syndrome manifested by the apes, nor were subsequent investigators more successful in confirming the onservations of Cantacuzene and of Bernhardt. From the failure to discover an organism of known characteristics, rather than from any positive evidence has grown the belief so generally held that the etiological agent of scarlatina is an ultramicroscopic virus.

During the many years that investigators have searched for the causative agent of scarlet fever, and with the varying emphasis attached to one or another species of parasite from time to time, the constant relationship to this disease of one organism S. hemolyticus has become more and more significant. As early as 1885 Crook (11) reported the presence of streptococcus in the blood and organs of individuals dying of scarlet fever. Loeffler (12), in addition, found this organism to be present in certain types of necrotic angina associated with scarlet fever and was furthermore successful in isolating the germ in pure culture. At this time Klein (13) likewise isolated a streptococcus from the tissues of patients with scarlatina, which he named Streptococcus scarlatinae. In 1885 the latter observer while investigating an outbreak of fever among certain cows belonging to a farm at Hendon, England, isolated from ulcerative lesions of the udders and from certain viscera, a streptococcus which he considered to be identical with Streptococcus scarlatinae. This observation was not only of great interest but also of very great impor-

tance because the milk obtained from the infected cows was shown to have been consumed by persons who subsequently developed scarlet fever. These early observations of the frequent relationship of streptococcus to scarlatina were soon confirmed by many students of the disease in different parts of the world. In 1900 Baginsky and Sommerfeld (14), reported the constant presence of streptococcus in the throat during the characteristic angina in seven hundred cases of scarlet fever. They also found this organism frequently in the blood, bone marrow and internal organs of patients dying of this disease. Other observers found streptococcus in the blood of fatal cases of scarlet fever in as many as 70 per cent of the individuals studied. Hektoen (15), furthermore, found the organism in blood in 12 per cent of patients during life, and his observations are of especial interest in that they indicate that the usual bad prognostic import of this phenomenon does not necessarily hold for scarlet fever.

In addition to the presence of streptococcus in the throat and blood of individuals with scarlet fever, this organism has also been proven to be the most frequent cause of the septic complications of the disease. Many times in septic foci streptococcus has been found in pure culture, and it is an old observation that convalescent individuals with discharging suppurative lesions are especially likely to give rise to return cases of scarlet fever showing that in such lesions the causative virus persists in an active form for long periods of time.

Such widespread and constant association of S. hemolyticus with scarlet fever has led some investigators to propose the view that streptococcus is the etiological agent of this disease. Certain observers, on the other hand, oppose this belief and have considered it more likely that streptococcus plays in scarlatina the rôle of a secondary invader. The objections of this latter group to the etiological significance of streptococcus are based upon certain important considerations. As is well known streptococcus is an organism of very widespread distribution and gives rise to a variety of pathological lesions such as abscess formation, cellulitis, septicemia and numerous other conditions. Frequently the same individual may have throughout life repeated streptococcus infections, one attack not seeming to confer immunity against subsequent invasion of the tissues by the same organism. The latter condition of affairs is especially true of

erysipelas, one of the most characteristic of the streptococcus diseases. On the other hand, scarlet fever, in sharp contrast to other streptococcus infections, is a fairly definite clinical entity and one attack appears to give rise to an immunity of life long duration. This peculiarity of scarlet fever might have been explained had it been possible to prove that the streptococcus associated with scarlet fever differed specifically from the hemolytic streptococci causing the various septic processes. However, early cultural and biochemical studies have failed to demonstrate any significant differential characteristics by means of which Streptococcus scarlatinae could be separated biologically from similar streptococci found in other diseases. When grown in fluid or in solid media, hemolytic streptococci resemble one another very closely, whatever be their source. It is true that certain constant differences can be brought out by means of fermentation of various carbohydrates, but such variations as exist apparently do not bear any specific relationship to a single disease process, and have been of but little aid in determining the etiological significance of streptococcus in scarlet fever. In addition to this objection Jochmann (16) has emphasized especially his failure to find streptococcus in either the blood or tissues of individuals dying in a few days from malignant forms of the disease. Since, therefore, types of streptococci indistinguishable from those seen in scarlatina are found in a great variety of disease conditions, and since the quality of the immunity in this disease differs widely in its duration from that observed in other streptococcus infections, and finally because of Jochmann's contention that streptococcus is not present in certain malignant types of scarlet fever, the conclusion has been drawn that streptococcus cannot be the cause of the disease.

An effort to meet these objections has been made by the group of investigators who believe that streptococcus is the etiological agent of scarlet fever. The observation by Baginsky and Sommerfeld of the constant presence of S. hemolyticus in the throats of all cases of scarlatina, an observation later confirmed by others, has done much to offset the inferences drawn from Jochmann's failure to find it in a few instances of fulminant types, especially since we now know that the organism in the latter cases may have been localized in some inaccessible area. Attempts were made in addition to explain the

immunity in scarlet fever and to establish the type specificity of the scarlatinal streptococcus. Moser (17) and Moser and Pirquet (18) have claimed that scarlatinal convalescent serum agglutinates to a higher titer Streptococcus scarlatinae than does control serum from other diseases. Furthermore, they have prepared polyvalent serum from horses, using the streptococcus of scarlet fever as antigen and have studied the capacity of such sera to agglutinate specifically various strains of scarlatinal streptococci. The latter strains were agglutinated in dilutions of 1:1000 or over, whereas hemolytic streptococci from other sources were not specifically agglutinated. As a consequence of these observations Moser and Pirquet believed that the streptococcus of scarlet fever differs specifically from apparently similar strains isolated from instances of erysipelas, phlegmon and puerperal sepsis. Meyer (19) and Rossiwall and Schick (20) have confirmed the results of Moser and Pirquet. Unfortunately, however, certain later studies by Hasenknopf and Salge (21), Aronson (22), and Neufeld (23) failed to support the earlier ones and grave doubt was thrown upon the specificity of Streptococcus scarlatinae.

Other interesting facts which indicate the specific relationship of streptococcus to scarlet fever have come from the studies of Gabritchewsky (24) on the specific prophylaxis of scarlet fever by means of a vaccine prepared from S. scarlatinae, and of Moser (25) on the therapeusis of the disease by means of a specific antistreptococcus serum. Gabritchewsky and his coworkers immunized a large number of individuals against scarlet fever with a vaccine prepared from hemolytic streptococci isolated from scarlatina. During the process of immunization certain phenomena occurred which were highly suggestive of the clinical manifestations of scarlet fever. In the majority of instances an area of erythema and swelling averaging 15 cm. in diameter developed at the site of the vaccine injection appearing in from eight to twenty-four hours and lasting about forty-eight hours. In general the erythema was diminished or absent following a second injection some ten days later. In about 15 per cent of the individuals inoculated a general reaction was observed. This general reaction consisted in fever of 1°C. or so, leucocytosis and an erythematous rash, having the characteristic distribution of the exanthem in scarlet fever. Some of those inoculated showed the

typical angina and strawberry tongue peculiar to the disease and in a few instances signs of renal irritation were observed. In general individuals who were recovering from the disease or who had had it some years before failed to show either a local or general reaction. Administration of Moser's antiscarlatinal serum before the inoculation was shown to prevent the development of both a local and a general reaction. Prophylactic immunization of this type seemed to diminish the incidence of scarlet fever among the inoculated. As a result of these observations Gabritchewsky and his assistants were strongly of the opinion that streptococcus is the causative agent of scarlet fever.

The therapeutic results obtained by the use of Moser's serum lent further support to this view. Moser immunized horses to hemolytic streptococci obtained from the blood of patients suffering from scarlatina. The serum thus prepared was used therapeutically and is said to have had marked beneficial effects causing a drop in the temperature and pulse, a diminution of the toxemia, early disappearance of the rash and a marked shortening of the duration of the disease. Escherich who observed the work closely was much impressed by the therapeutic value of the serum and likened its action to that of diphtheria antitoxin. Later antistreptococcic sera prepared by other investigators, however, failed to display the therapeutic efficiency of Moser's serum and created doubt in the minds of many concerning the usefulness of such sera.

Much other evidence both for and against the etiological relationship of streptococcus to scarlet fever was presented at this time and as one weighs its importance in retrospect, the positive seems of more significance than the negative. The outstanding objection, however, to the acceptance of streptococcus as the cause of scarlet fever remained the impossibility of differentiating satisfactorily this organism from hemolytic streptococci associated with the great variety of septic conditions. As a result other etiologic agents were searched for. Moser's serum dropped into disuse and streptococcus vaccine was no longer used in the prophylaxis of scarlet fever. Scientific opinion gradually came to hold that streptococcus bore an important but secondary relationship to scarlet fever, and that the true cause must be sought among the unknown viruses.

For many years confusion has existed and opinion has varied concerning the existence of biologically varying types of streptococcus. Two diverging points of view developed, one maintaining the unity of the species as a type, and the other holding that it comprised a group of organisms different from one another in their biological characteristics. Schottmüller (26) in 1903 made an important contribution to the discussion in demonstrating between certain streptococci, differences based on their action on blood agar plates, one group hemolyzing the red blood cells and the other either failing to hemolyze or forming methemoglobin. This significant differentiation resulted in the establishment of the types now generally recognized as hemolytic and nonhemolytic or green pigment producing strains. Further classification was attempted by numerous investigators who used as a basis of differentiation certain biochemical reactions. Holman (27) in 1916 using carbohydrate fermentation as a test was able to demonstrate the existence of a number of separate fermentation types. Numerous efforts were also made to establish biological differences, especially among the hemolytic streptococci, by means of serological reactions methods which had proven singularly successful when employed for studying the various types of pneumococcus and meningococcus. As a result of these studies conflicting beliefs arose, and a definite opinion could not be given as to whether or not separate biological types of S. hemolyticus exist. As late as 1918 Swift and Kinsella (28) using the complement fixation reaction as a test made a series of observations of twenty-eight strains of hemolytic streptococcus from various sources. They found that they were unable to determine significant serological differences between the strains studied and are of the opinion that a striking homogeneity exists. Efforts to correlate such different types of hemolytic streptococcus as had been determined with specific pathological lesions were also of indeterminable significance, varying types being found in association with the same disease.

In 1918 Dochez, Avery, and Lancefield (29) undertook a biological study of a great number of strains of S. hemolyticus, obtained from a variety of pathological conditions among the changing population of a large military establishment. The purpose of this investigation was to determine if there exist among the hemolytic streptococci diverse

biological types as is the case in the instances of pneumococcus and meningococcus. The specific test reactions were those of agglutination and protection. Spontaneous non-specific flocculation, the most confusing factor in previous studies of specific agglutination of streptococcus, was avoided by the employment of special methods. The outcome of these studies was to prove that there are separate biological types among hemolytic streptococci, just as there are among other apparently closely related groups of microörganisms. More than 68 per cent of the strains investigated comprised six easily distinguishable serological types.

This study was part of a general investigation of the biology of streptococcus, and, as a result of the facts developed, Bliss and Dochez (30) undertook a reinvestigation of the much debated question of the unity of type of the S. hemolyticus so constantly associated with scarlet fever. An effort was made to answer Jochmann's main objection to the etiological relationship of S. hemolyticus to scarlet fever, namely, that the organism is not present in every instance of the disease, and that it cannot be satisfactorily differentiated from hemolytic streptococci associated with the common septic conditions. Bliss (31) found when cultures are made from the throat early in the course of scarlet fever that hemolytic streptococci are present in predominating numbers in 100 per cent of individuals examined, thus confirming the earlier work of Baginsky and Sommerfeld. Immune sera were then prepared by the inoculation of rabbits with scarlet fever streptococci, and the capacity of these sera to agglutinate specifically a large number of freshly isolated scarlet fever strains was tested. Ten such sera were prepared from different strains of scarlet fever streptococci and each serum was found to agglutinate more than 80 per cent of the strains isolated from scarlatinal throats. Agglutinating sera prepared from strains of hemolytic streptococci derived from pathological sources other than scarlet fever in general, failed to agglutinate specifically the scarlatinal strains. Furthermore, strains of hemolytic streptococci obtained from such conditions as tonsillitis, erysipelas, broncho-pneumonia, and other septic diseases, as well as the various type streptococci, determined by Dochez, Avery and Lancefield were not agglutinated by the scarlatinal antistreptococcic sera. The evidence in favor of the

specificity of the agglutination reaction of scarlatinal streptococci was reinforced by results obtained from agglutinin absorption experiments. Scarlatinal streptococcic sera also afforded some protection of experimental animals against virulent scarlet fever streptococci, but had no protective power against hemolytic streptococci from other sources. This work indicates that the majority of hemolytic streptococci found in association with scarlatina belong to a specific biological group and can by appropriate methods be distinguished from hemolytic streptococci derived from other pathological conditions. These observations, I believe, confirm in a satisfactory manner the early studies of Moser and von Pirquet on the same subject. Contemporaneously with Bliss, Tunnicliff (32) investigated, by means of the opsonic and agglutination reaction, a series of hemolytic streptococci isolated from patients during the early stages of scarlet fever. She concludes that the serum of sheep immunized with hemolytic streptococci from the throat in the acute stage of scarlet fever contains opsonins and agglutinins for the hemolytic streptococci that prevail in the throat and complicating lesions early in this disease, but not for hemolytic streptococci from other sources, such as erysipelas, mastoiditis, measles, influenza, diphtheria and the normal throat. The results of her absorption experiments also indicate that the hemolytic streptococcus from scarlet fever forms a distinct group, scarlatinal streptococci removing the opsonins and agglutinins for these cocci while absorption with a hemolytic streptococcus from erysipelas has no such effect. These results also suggest that the hemolytic streptococci from scarlet fever form a distinct serologic group. Somewhat later Gordon (33) found that eighteen strains of hemolytic streptococcus isolated from scarlatina were identical in their agglutinative reactions. None of these strains absorbed the agglutinins from immune sera prepared from certain other types of hemolytic streptococcus, designated by him as Types I and II. On the basis of this evidence, Gordon concludes that the streptococci from the throat secretions in scarlet fever constitute a group immunologically distinct from other varieties of streptococcus pyogenes. Eagles (34) in a recent study compared the serological reactions of hemolytic streptococci from scarlet fever, puerperal sepsis, erysipelas and miscellaneous sources. He confirms

the immunological specificity of the scarlatinal group and the clearness with which it can be separated from other types of streptococcus. He furthermore compared in an interesting manner, a number of individual strains obtained at three to four day intervals from the same patient and demonstrated a gradual but progressive loss of specific agglutinability, a phenomenon which we have observed, and of which I shall say more later. Williams (35) has also studied the serological reactions of the scarlatinal streptococci and finds only 35 per cent to belong to a single type, and is of the opinion that a greater variability exists than is suggested by the work of the previous observers. Dick and Dick (36) have shown two strains of scarlet fever streptococci, one a mannite fermenter, and the other a non-mannite fermenter, to be serologically distinct, and believe that the agglutination reaction is of but little importance in determining the character of the scarlatinal streptococci.

It would seem, therefore, that the old question stressed by Jochmann, concerning the specificity of the streptococcus of scarlet fever still remains in dispute. The preponderance of evidence, however, strongly favors the belief that these cocci comprise a separate biological group and that the best method for determining this specificity of type is by agglutination. In order that satisfactory results may be obtained from this reaction certain rigid conditions must be complied with; spontaneous autoagglutination must be prevented, and the streptococci in question must be studied fresh from their human environment. This latter requirement is of great significance. Recent studies by Avery and Heidelberger (37) have shown that the type specificity of pneumococcus is dependent upon the chemical constitution of the capsular substance. The production of this substance is a variable function of the organism; it is greatest in its strictly parasitic phase and is reduced by all factors which reduce virulence and lessen pathogenicity. That a similar loss of a specific function by scarlatinal streptococci takes place when they are removed from their parasitic environment is extremely likely. Bliss and Stevens and Dochez (38) have emphasized the rapidity with which specific agglutinating qualities are lost upon continued growth of these streptococci in artificial medium and Eagles suggests that the same change may take place under the influence of the im-

mune bodies formed by a scarlatinal subject during convalescence. The suppression of specificity of serological reaction under the influence of immune bodies is of course a well recognized and established phenomenon among the pneumococci. The results obtained therefore indicate that if a large number of strains of scarlatinal streptococci are studied under appropriate conditions and within a short period of time from their isolation during the acute stage of scarlet fever a high degree of serological specificity can be demonstrated.

Streptococcus scarlatinae is found not only in the throats and organs of individuals suffering from anginal types of scarlet fever but has also been obtained from atypical forms of the disease, healthy carriers and contaminated food products. Serologically specific streptococci have been isolated from the local lesions in scarlet fever arising from the infection of wounds and burns, from the throat in scarlet fever without a rash, and from the lochial discharge in instances of puerperal scarlet fever. Bliss succeeded in tracing a small outbreak of scarlatina in an isolated children's institution to a recently admitted healthy carrier of Streptococcus scarlatinae. Stevens and myself identified by means of agglutination and absorption reactions as scarlatinal streptococci organisms isolated both from the contaminated milk which had given rise to a milkborne epidemic of scarlet fever, and from the throats of patients who contracted the disease from this milk. As a result of these studies the importance of Jochmann's objections to Streptococcus scarlatinae as the etiological agent of scarlet fever was much lessened and students again began to take an active interest in this organism as the probable cause of the disease.

From the very beginning of the study of scarlet fever efforts have been made to produce the disease experimentally in animals and in man by inoculation with scarlatinal material. Most of these attempts have had in view the demonstration of an unknown virus of the filter passing type. Streptococcus scarlatinae in spite of the presumptive evidence in its favor and of the fact that some of the most typical examples of scarlet fever in animals have been associated with its presence has been but little tested for its capacity to produce the disease experimentally. Class (39) in 1899 reported the experimental production of this disease in swine by an organism designated

by him as Micrococcus scarlatinae. This was a gram negative coccus isolated from three hundred cases of scarlet fever and was in all probability a streptococcus. Krumwiede, Nicoll and Pratt (40) in 1914 observed an accidental infection of a laboratory worker, who sucked into her mouth a mixture of living streptococci containing Streptococcus scarlatinae. Three days later this individual developed a sore throat and subsequently experienced a typical attack of scarlet fever with all the usual phenomena. Because of the interest aroused by this observation, efforts were made to infect monkeys with the same streptococcus but no instance of the disease was successfully produced.

In 1921 Dick and Dick (41) made a series of human inoculations with certain organisms obtained from the throats of individuals suffering from scarlet fever. Among the organisms utilized for this purpose was Streptococcus scarlatinae. Though some of the volunteers experienced sore throats as a result of the treatment no true instance of experimental scarlet fever resulted. In 1923 the same workers (42) repeated their efforts to produce scarlet fever in human volunteers. In the second series of observations a hemolytic streptococcus obtained from the infected finger of a nurse suffering from wound scarlet fever was used for purposes of inoculation. Five volunteers were inoculated by swabbing the tonsils and pharynx with four-day-old cultures of the streptococcus in question. Three of these individuals remained without evidence of infection and one suffered from sore throat and fever without a rash. The fifth volunteer, however, who had been inoculated with the streptococcus after three weeks growth in artificial medium experienced a typical but mild attack of scarlet fever, beginning forty-four hours after inoculation, and characterized by sore throat, general malaise, nausea, fever, leucocytosis, a typical rash and albuminuria. Desquamation began on the hands and feet on the tenth day and was complete by the end of the fourth week. Five volunteers inoculated with filtrates of the above mentioned organism remained well and showed neither sore throat nor rash. Subsequent inoculation of four of these volunteers with living unfiltered cultures of the original streptococcus resulted in the experimental production of another instance of scarlet fever. These observations were confirmed later by the same inves-

tigators (43) by the experimental production of another instance of scarlet fever in an individual proven susceptible by the use of a skin test devised by them.

In 1920, Dochez and Bliss while studying the biological reactions of Streptococcus scarlatinae, observed in a dog infected subcutaneously with living organisms, the development of an intense general erythema followed later by desquamation. Attempts to reproduce this phenomenon in dogs resulted in failure. Stevens and Dochez later tried other animals, including monkeys without success. Failure in these instances seemed to be due to our inability to induce a local infection because of the low virulence of the organism for the animals employed. Finally Dochez and Sherman (44) were successful in producing in guinea pigs and young swine a series of manifestations comprising some of the principal phenomena of scarlet fever. Successful local infection was achieved by injecting melted agar subcutaneously and infiltrating the mass with living cultures of Streptococcus scarlatinae. Since it had become increasingly evident that scarlatina has a certain resemblance to diphtheria, in that there is a local infection in the throat from which the specific toxic substance is distributed, we hoped that a similar absorption of toxic material would take place from the local area of infected agar. This proved to be the case and guinea pigs and swine treated in the manner described developed an erythematous rash, fever, leucocytosis and progressive loss of weight. From eight to twelve days following infection the swine had general scaly desquamation and the guinea pigs slight general desquamation and complete separation of the skin over the pads of the feet. This phenomenon could not be induced when hemolytic streptococci from sources other than scarlet fever were utilized. Some of the guinea pigs died acutely from the toxic substances absorbed from the locally infected area, and after death streptococci could not be demonstrated by culture either in the blood or serous cavities.

The production of experimental scarlet fever in human beings and in animals by inoculation with Streptococcus scarlatinae had by this time made it increasingly likely that this organism is the causative agent of the disease. The evidence in favor of the absorption from the area of local infection of a toxic substance which might

be responsible for the clinical picture, had again brought into the foreground the analogy with diphtheria.

Investigators of scarlet fever have for many years been impressed with the similarity of this disease to diphtheria. Berge (45), as early as 1895, suggested that scarlatina is due to a local infection in the throat with streptococcus and that the general symptoms of the disease are due as in diphtheria, to the absorption into the general circulation of soluble toxins formed by the infecting microörganism at the site of the local disease. Gabritchewsky and his co-workers, in their studies of scarlatiniform manifestations which followed immunization of human beings against scarlet fever by means of vaccines of killed cultures of streptococcus scarlatinae, attributed these reactions to the presence of a toxin in the vaccines. They drew attention to the absence of a vaccine erythema in individuals who gave a history of having had scarlet fever, and its failure to develop in patients during the period of convalescence from this disease.

Much evidence in favor of the existence of a soluble circulating poison in scarlet fever has also come from the study of the so-called Schultz-Charlton extinction phenomenon. In 1918 Schultz and Charlton (46) discovered that if one injects into the skin of a scarlet fever patient with a bright red rash 1 cc. of serum from a normal person, or from a patient convalescent from scarlet fever, there appears after a time at the site of the injection a characteristic change. This change begins after about six hours and consists in a complete blanching of the rash over an area of from one-half inch to a few inches in diameter. In the affected area the swollen follicles, which are a feature of many rashes, disappear. Looked at from a distance the margin of the defect in the rash is generally sharply defined. The colour of the blanched area is that of normal skin and the duration of the typical phenomenon coincides on the whole with that of the rash itself. On the other hand, serum taken from scarlet fever patients during the acute stage of the illness invariably gave negative results. Subsequent investigators abundantly corroborated the accuracy of the observation of Schultz and Charlton. As a result of these later studies it was established that the serum of about sixty per cent of normal adults possesses the capacity to blanch the rash in an active case of scarlet fever; that convalescent scarlatinal serum

gives a positive rash extinction test in from 80 to 100 per cent of instances; and that the serum during the active stages of scarlet fever never manifests blanching power. The Schultz-Charlton reaction was first used as a diagnostic test of scarlet fever, and the capacity to extinguish the rash in scarlet fever was believed to be due to a normal property of human serum, which is temporarily lost during the acute stage of scarlet fever and regained during convalescence.

In 1923 Mair (47) published a study of the Schultz-Charlton reaction in which he confirmed in general the observations of previous workers but gave the phenomenon a much more satisfactory explanation. He had an opportunity of studying the blanching power of the serum of a child both before and after an attack of scarlet fever and showed that the serum before the attack gave a negative Schultz-Charlton test but during convalescence acquired the capacity to extinguish an active rash. This disproved the previous belief that a positive reaction was due to some property of normal human serum which is lost during the acute stages of scarlet fever. He also showed that the sera of young children who had not had scarlet fever, give a negative reaction in a much greater proportion of instances than do adult sera and that the reactivity of the sera of newborn infants corresponds with that of the mothers.

Mair had been interested for some years in the resemblance of scarlet fever to diphtheria. As a result of his later work he came to believe that the rash and other changes in the skin in scarlet fever are due to a scarlatinal toxin which has entered into combination with the tissue cells. Among the affected cells are those contractile elements which have been shown to exist even in capillary blood vessels, and to the function of which the normal tone of the capillaries is due. The toxin interferes with the function of these cells and a loss of tone of the capillaries results in the erythema and exudative phenomena with which we are familiar in the scarlatinal rash. He supposes that the serum giving a positive Schultz-Charlton reaction contains an antitoxin which is able to dislodge and neutralize the toxin fixed in the cells, and thus restores their normal function over the area injected. He adds that the true causal organism when discovered should be capable of producing a toxin, and that the immunization of animals to this poison should give rise to an antitoxin capable of producing a positive Schultz-Charlton reaction in man.

We also had been pondering over the analogy between scarlet fever and diphtheria, and, at the time of the publication of Mair's observations, had already produced in horses by immunization to Streptococcus scarlatinae an antitoxic serum of the type postulated by him. Struck by the fact that occasionally in guinea pigs inoculated for the production of experimental scarlet fever sufficient poison was absorbed from the local lesion to kill the animals acutely, we determined to make use of the method for the production of an antitoxic serum in horses. Masses of melted nutrient agar were injected beneath the skin and then infiltrated with increasing doses of Streptococcus scarlatinae. The animals experienced a general reaction and some of them curiously enough showed loss of hair and extensive general desquamation. After nine months immunization the first animal was bled and his serum tested by Blake and Trask and Lynch (48) for its correspondence with human convalescent scarlatinal serum. When injected intracutaneously in a patient with a bright rash in the acute stage of scarlet fever this serum caused a complete extinction of the rash over an area five to ten centimeters in diameter. The blanching appeared in from six to twelve hours following injection of the serum and persisted throughout the course of the disease. As a rule the characteristic pigmentation and desquamation were absent during convalescence over the blanched area. Antisera prepared from other hemolytic streptococci and from Streptococcus scarlatinae injected intravenously into animals, failed to induce blanching of the rash. Furthermore, scarlatiniform rashes in such conditions as erysipelas, measles, and other exanthematic diseases, were not influenced by the intracutaneous injection of the scarlatinal antitoxin. Injection of a sufficient quantity of the serum intramuscularly in a patient in the exanthematous stage of scarlet fever causes a complete fading of the rash over the whole body in from twelve to twenty-four hours.

Blake and Trask (49) have demonstrated that there is present in the circulating blood and in the urine during the acute stage of scarlet fever a toxic substance which causes an erythematous reaction when injected intracutaneously in individuals whose blood serum gives a negative rash extinction test. This substance appears to be identical with the culture toxin of the Dicks and circulates in the

blood for several days. When patients with scarlet fever having easily demonstrable amounts of this poison in the blood are injected with scarlatinal antitoxin, the circulating toxin is rapidly neutralized, a single dose of forty cubic centimeters causing its complete disappearance throughout the remaining course of the disease. The antitoxin quickly predominates in the blood and the treated patients' serum acquires the capacity to induce a positive Schultz-Charlton extinction test, a property that does not develop in untreated patients until late convalescence. The other toxic manifestations of the diseases are likewise favorably influenced. An immune horse serum, therefore, prepared in the manner described, seems to contain a potent antitoxin and behaves in every way in a manner similar to human convalescent scarlet fever serum.

The further studies of Dick and Dick (50) demonstrating the presence of a toxic substance in filtrates from blood broth cultures of Streptococcus scarlatinae have brought to light a number of new and important facts which develop still further the analogy between scarlet fever and diphtheria. The toxic filtrate was obtained by these authors from a strain of streptococcus with which they had produced experimental scarlet fever in man. When individuals who give a negative history for scarlet fever are injected intracutaneously with small amounts of this toxin, within about six hours there appears at the site of inoculation a small circular area of erythema, which increases in size and intensity of color for from eighteen to thirty-six hours. Frequently the local reaction is accompanied by swelling of the skin. When a series of normal persons who have not had scarlet fever are injected with this substance, 41.6 per cent of these show a positive erythema reaction in the skin, a manifestation resembling the Schick test for susceptibility to diphtheria. The remainder who give a negative reaction are considered to be immune, because of the probable presence of circulating antitoxin in the blood, just as in the case of diphtheria. In addition, patients who are recovering from scarlet fever when tested intracutaneously with this substance, give but a very faintly positive or uniformly negative skin reaction. A similar condition of affairs is found to exist among those who have had scarlet fever at some earlier period of life. If individuals who have been proven susceptible to scarlet fever by means

of the Dick test, are injected subcutaneously with larger amounts of the toxin, they exhibit certain of the toxic manifestations of the disease, such as nausea and vomiting, fever and an erythematous rash. When toxic filtrate is mixed *in vitro* with a small amount of convalescent scarlet fever serum, its capacity to produce a positive skin reaction is completely neutralized. Neutralization of the reaction was also obtained *in vivo* by the injection into susceptible human beings of larger quantities of convalescent serum. More recent studies of the Dicks (51) have shown that individuals who react positively in the skin, can be immunized by repeated doses of the toxin, so that within a relatively short period of time the skin reaction becomes negative and there is some evidence to support the belief that such individuals may be immune to the disease scarlet fever.

Zingher (52) in an extensive study has confirmed the observations of the Dicks and extended them somewhat. He has shown that the Dick reaction is positive in the early stages of scarlet fever in most instances, and that it becomes increasingly negative as the disease progresses through convalescence. He has, furthermore, drawn a very close analogy between the data obtained from the Schick test in diphtheria and those obtained from the Dick test in scarlet fever. In general, susceptibility to the latter reaction is greater in childhood and diminishes in adult life. There is also an inherited resistance to the toxin in infants whose mothers exhibit a negative reaction.

These studies, therefore, indicate that there is present in sterile filtrates from cultures of Streptococcus scarlatinae a toxic substance which bears a specific relationship to scarlet fever. By means of this substance it is possible to detect susceptibility in persons who have not suffered from scarlet fever, and furthermore to demonstrate the development of immunity in patients who are recovering from an attack of the disease. This work brings further strong support to the belief that Streptococcus scarlatinae is the etiological agent of scarlet fever.

In 1921, Di Cristina (53) in Italy, obtained from the blood of patients with scarlet fever an anaerobic Gram-positive diplococcus. Other Italian investigators subsequently isolated a similar organism

from the naso-pharynx, bone marrow, spleen and desquamating skin of children with scarlet fever. This organism, on further study, was found to show specific serological reactions with the serum of recovered cases of scarlatina. Inoculation of children with living cultures of the organism is said to have produced an attenuated form of scarlet fever. Furthermore, prophylactic inoculation with killed cultures prevented the development of scarlet fever among a number of children exposed to the disease. Unfortunately, we are not in a position as yet to determine with any assurance the significance of this organism in scarlet fever, since an opportunity to study it bacteriologically has not been afforded.

Have we now reached the end of man's long struggle to find the cause of this interesting and at times formidable and dangerous disease? Personally, I think we have. Belief that scarlet fever may be caused by a protozoan parasite, or by one of the mysterious ultramicroscopic viruses, must I think be discarded in view of the fact that the evidence brought forward in support of the causative relationship of such types of microörganisms to the disease is entirely unconvincing. On the other hand, can we say with certainty that scarlet fever is caused by a type of Streptococcus hemolyticus? Certainly a chain of evidence in favor of this organism has been patiently and progressively forged which is as strong as that in many diseases whose etiology is now accepted without discussion. The constant association of this organism with the primary and secondary manifestation of the disease, its specific character, its capacity to produce the experimental disease in man and in animals, the quality of human convalescent scarlet fever serum to neutralize the toxic effects of this streptococcus, the capacity of an antistreptococcus horse serum antitoxic in nature to counteract the specific toxic manifestations of the disease in man, and finally the isolation from Berkefeld filtrates of this streptococcus of a toxic substance which bears a specific relationship to immunity in scarlet fever, leaves little room to doubt that Streptococcus scarlatinae is the principal and probably only etiological agent of scarlet fever.

Let us, therefore, be optimistic and assume that a just reward has come to those many soldiers in the army of science, too numerous to be mentioned in so short an exposition, and that another disease

has been added to those about which the essential specific facts are known. Let us also hope that the methods of prevention and treatment based on these facts may prove as successful as the promising character of the preliminary work suggests.

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HYDROGEN ION CONCENTRATION OF THE BLOOD IN HEALTH AND DISEASE

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INTRODUCTION

The following discussion of the theoretical and practical considerations involved in the measurement of the hydrogen ion concentration and its significance in body fluids is intended only as a convenient outline for those readers whose interests have been clinical rather than physiological. The electrolytic theory is utilized in its simplest form, regardless of the present question of complete and partial dissociation and we use the familiar term hydrogen ion concentration rather than the less familiar though more accurate term, activity. Only at one point in the paper have we used the term "activity"; namely in referring to the Donnan equilibrium. In this instance it seems to us of especial importance to keep in mind the distinction between thermodynamic activity and chemical concentration.

In aqueous solution certain substances, acids, bases and their salts, are changed to greater or less degree, into components which carry an electric charge which are called ions. This process of electrolytic dissociation may be represented by the equation

$$HA \rightleftarrows H^+ + A^- \qquad (1)$$

This phenomenon is of the utmost importance in life processes and especially so in the complex mammalian organism because the proc-

esses of digestion, respiration and excretion are all to a great extent concerned with substances which in solution dissociate into electrolytes. Of immediate interest to this discussion are the acidic and basic substances which are present in the body fluids.

Two points are of especial importance: (1) that this reaction is a reversible one and that the undissociated molecules are in continual equilibrium with the ions, and (2) the ions as well as the undissociated molecules for the purposes of this discussion may be assumed, in the concentration met with in blood, to obey the law of mass action.

Measure of acidity in solutions

In measuring the alkalinity or the acidity of any solution two factors must be considered, the *quantity* of the acidic or basic substance and the *intensity* of the degree of acidity or alkalinity. The quantity factor is expressed in terms of the concentration of the substance in chemical terminology as the normality or the number of gram equivalents per liter.

The *intensity* factor, the degree of acidity or alkalinity, often referred to as the *reaction* of the solution, depends upon the H^+ and OH^- ion concentration and is expressed usually in terms of the normality of hydrogen *ions*.

A most important property of aqueous solutions is that the concentrations of H^+ and OH^- ions are related to each other thus:

$$[H^+] \times [OH^-] = K_w \qquad (2)$$

where for any given temperature K_w is a constant and when $[H^+]$ increases $[OH^-]$ decreases and vice versa, so that it is possible at any one temperature to express both acidity and alkalinity in terms of either ion. It is usual to use the $[H^+]$ for this purpose and to speak of the hydrogen ion concentration of the solution. Throughout this paper we will use a bracketed chemical symbol to indicate the concentration (or activity) of a substance in solution.

The relation between the intensity and the quantity factor of acidity is analogous to the relation between the intensity and quantity factors in electrical energy. Electromotive force is the intensity factor which determines whether or not current flows and the quantity factor, ampère, is the measure of the total amount of current. This

relation may also be compared to differences between temperature and calories, or to the older analogy between the pressure of water, due to the height of the reservoir, and the content of the reservoir.

Another interesting comparison between the intensity factors $[H^+]$ and t° is that the zone of $[H^+]$ compatible with life is as small compared to known $[H^+]$ concentrations as is the zone of temperature compatible with life to known temperatures.

In biological solutions the normality of $[H^+]$ is so small that the fractional values are difficult to visualize. Furthermore, in determining and plotting $[H^+]$ values it is often convenient to use logarithms. Soerensen (1909) therefore introduced the term pH as a convenient symbol for a measure of $[H^+]$ concentration, defined as follows:

$$\mathrm{pH} = \log\left(\frac{1}{[H^+]}\right)$$

The relation of pH to $[H^+]$ is most clearly shown by examples. An N/10 HCl solution, assuming it to be completely dissociated into H^+ and Cl^- ions, will also be tenth normal with respect to hydrogen ions, or

$$[H^+] = 1/10\ \mathrm{N} = 0.1\ \mathrm{N} = 1 \times 10^{-1}\ \mathrm{N}$$

or

$$\text{its pH} = 1.0$$

In like manner the hydrogen ion concentration of a serum may be expressed thus

$$H^+ = 0.000{,}000{,}032\ \mathrm{N} = \frac{0.32}{10^7}\ \mathrm{N} = 0.32\ \mathrm{N} \times 10^{-7}\ \mathrm{N} = \frac{1}{10^{7.5}}\ \mathrm{N} = 10^{-7.5\mathrm{N}}$$

or

$$\text{its pH} = 7.5$$

In using pH it is necessary to remember that change of 1 pH unit indicates a 10-fold change in H^+ concentration, that increase in pH means decrease in $[H^+]$ and that pH 7.0 at 20° and 6.8 at 38° represent neutrality. Thus a decrease of pH from 7.4 to 6.4 means that the H^+ concentration has become 10 times *greater*, and that at either 20° or 38° the solution instead of being slightly alkaline, has become slightly acid.

Relation between [H^+] and gram equivalent normality of acid concentration

Since [H^+] is the measure of the *intensity* factor, and titration of the *quantity* factor, it follows that for any solution the difference between these two measurements is dependent upon the degree of dissociation. With an almost completely dissociated acid, as tenth or hundredth normal HCl solution, the two measurements expressed in normalities agree but in the case of many acids as carbonic, phosphoric, acetic, etc., the dissociation is low and the [H^+] normality is therefore only a small fraction of the gram equivalent normality of total acid concentration.

In such solutions therefore the titration values give no indication of the actual [H^+] concentration of the solution.

Relation of [H^+] to buffer effect

Let us now compare the effect of adding strong acid to solutions of salts of strongly and weakly dissociated acids. If we add 1 cc. of N/10 HCl to (1) a liter portion of 0.15 N sodium chloride (physiological saline) and (2) to a liter portion of 0.15 M sodium phosphate solutions both at neutrality (pH = 7), the resulting pH of the NaCl solution will become about 4, a 1000-fold increase in acidity, while the pH of the phosphate solution will be practically unchanged. The importance in biology of substances, whose solutions show comparatively small changes in [H^+] with large addition of acid or alkali was pointed out by L. J. Henderson (1908) and to them Soerensen (1909) gave the name "buffer" substances.

The hydrogen ion concentration of body fluids in general and of the blood in particular is kept within rather narrow limits by means, in large measure, of such buffer systems. In the blood the systems are in order of importance, hemoglobin acid and its salt, carbonic acid and alkali carbonate, serum proteins and their salts, and monobasic and dibasic phosphate. In this discussion the proteins including hemoglobin will be considered to be dissociated as weak acids (see Loeb, 1922). The [H^+] of a mixture of a weak acid with its salts may be calculated by the equation

$$[H^+] = K\frac{[\text{free acid}]}{[\text{salt}]} \qquad (3)$$

Thus for serum

$$[H^+] = K_1 \frac{[H_2CO_3]}{[BHCO_3]} = K_2 \frac{[BH_2PO_4]}{[B_2HPO_4]} = K_3 \frac{[\text{H protein}]}{[\text{B protein}]} \quad (4)$$

where K_1 K_2 K_3 represents the dissociation constants for the individual systems and B represents sodium or potassium.

L. J. Henderson (1908) first showed that the carbonate and phosphate equilibria of this equation are applicable to the blood system. In 1916, Hasselbalch used the equation in the logarithmic form and gave values for pK′ in the following equation:

$$pH = \log \frac{1}{[H^+]} = \log \left(\frac{1}{K \frac{[H_2CO_3]}{[BHCO_3]}} \right) = \log \left(K' \frac{[BHCO_3]}{[H_2CO_3]} \right) = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]} \quad (5)$$

Several relations, of especial importance in blood may be deduced from the above equations. First, for a given $[H^+]$ the *ratios* of acid and salt components to each other are fixed for every system. Secondly, if the ratio for any one system can be determined and if the K values are known the value of $[H^+]$ as well as of all the other ratios can be calculated. This fact is the basis for the calculation of pH or of pCO_2 or $[H_2CO_3]$ when one of these and total $[CO_2]$ are known.

A third fact is evident from (4): that $[H^+]$ is not dependent upon the concentration of the buffer substance but upon the ratio of concentration of its two components. This is discussed more fully under "Alkali reserve."

The acid base balance of the blood

The reaction of the blood is maintained within narrow limits by a remarkable adjustment to varying conditions. The daily metabolism involves the ingestion of varying quantities of acid and basic salts, the production in metabolism of tremendous quantities of acids and bases, espcially H_2CO_3 and NH_3 and the neutralization and excretion of these products. The physiological and chemical mechanisms of these processes have been reviewed thoroughly recently by L. J. Henderson (1921), Van Slyke (1921a, 1921b), Barcroft *et al.* (1922) and Wilson (1923) so that we need only refer briefly to factors which are of importance in abnormal conditions.

Under normal conditions the reaction of the blood is stabilized

through three main processes in addition to the effects of its buffer: excretion of the non volatile acids and bases through the kidney, change in the base binding properties of hemoglobin with oxygenation and reduction, and excretion of carbon dioxide through the lungs.

The first process is slow compared with the almost instantaneous adjustments secured by the last two.

The amounts of CO_2 formed in the tissue and transmitted to the circulating fluid is so great that if only the buffer action of the blood's buffer systems were available the resulting reaction would pass beyond the limits of acidity compatible with life. However, oxyhemoglobin is a stronger acid than reduced Hb so that as Hb is reduced, which occurs coincidently with increase of CO_2, some of the base B bound with it as $BHbO_2$ may combine with H_2CO_3. This process is reversed in the lungs, thus:

$$\text{Tissue: } BHbO_2 + H_2CO_3 \rightarrow BHCO_3 + HHb + O_2 \quad (6)$$

$$\text{Lung: } BHCO_3 + HHb + O_2 \rightarrow BHbO_2 + H_2CO_3 \quad (7)$$

By such a mechanism the ratio of $\frac{H_2CO_3}{BHCO_3}$ is kept approximately constant (within 0.03 pH) and at the same time the transfer of oxygen is aided. That each of these processes facilitates the other was pointed out by L. J. Henderson (1921).

From the viewpoint of acid base balance it is important that a certain amount of base is in a labile condition and can be exchanged between CO_2 and Hb in respiration.

In the serum this interchange may take place directly, but since neither sodium nor potassium diffuses through the cell wall, to any appreciable extent in this exchange, the exchange between cells and serum must be indirect. It is accomplished by the transfer of Cl^- and H^+ ions between cell fluid and serum fluid. Thus:

$$\overbrace{KCl + HHb + O_2 \rightleftarrows KHbO_2 + HCl}^{\text{cell}} \rightleftarrows \overbrace{HCl + NaHCO_3 \rightleftarrows NaCl + H_2CO_3}^{\text{serum}} \quad (8)$$

Alkali reserve

The importance of the system $\frac{[H_2CO_3]}{[BHCO_3]}$, in serving as the first line of defence against abnormal acids, lies in the ease with which excess

H_2CO_3 may be excreted through the lungs. As has been pointed out in discussing equation (5) the concentration of the system $\frac{[H_2CO_3]}{[BHCO_3]}$ may be changed so long as the ratio is constant, without change in pH. Thus whenever base is needed for neutralization of a stronger acid HX it can be furnished by the $BHCO_3$.

$$BHCO_3 + HX = BX + H_2CO_3 \qquad (9)$$

and the freed H_2CO_3 can be removed by increased respiration. Excess of alkali may be met by the reverse process of decreased CO_2 elimination. The $BHCO_3$ concentration in normal venous blood is maintained at between 45 and 60 vols. per cent of CO_2. The base of this $BHCO_3$ constitutes the alkali reserve against abnormal acid. The determination of the $[CO_2]$ of blood therefore serves as the most convenient means of measuring this alkali reserve. Since the amount of total CO_2 thus bound is dependent upon the pCO_2, Van Slyke and Cullen (1917a) used the CO_2 capacity (the total CO_2 content after exposure to normal pCO_2) as the measure of the alkali reserve. More recently Van Slyke (1921b) and the present authors have expressed the alkali reserve as the $BHCO_3$ content at constant pH.

CO_2 absorption curves

The influence of the total buffer systems of blood is most clearly shown graphically by CO_2 absorption curves. Two types of curves are generally used. One is with $[BHCO_3]$ or total $[CO_2]$ as the ordinate and pCO_2 as abscissae. Such a curve, first used by Bohr was employed in a very convenient graph by Haggard and Henderson (1919) giving both pH and $[H_2CO_3]$ values in addition.

In such a graph the difference in $[BHCO_3]$ with change in CO_2 tension is the measure of the buffer exchange of base with change in pH.

For many purposes a graph using $[BHCO_3]$ and pH as coördinates is preferable. Such a diagram is that shown in figure 5 which is adopted from Van Slyke's (1921b) chart. We have added to Van Slyke's diagram lines indicating CO_2 tension (Cullen and Jonas, 1923).

These curves indicate at once various conditions possible in the blood acid base system. Henderson, Bock, Field and Stoddard (1924) have recently given examples of many possible combinations of curves useful in studying the blood electrolyte equilibrium.

Influence of salts and proteins on the acid base equilibrium

The salt systems constituting the buffer systems in the blood comprise only one-fifth of the total salt concentration of the serum or blood. The total salt concentration of normal serum is equivalent to a NaCl solution of from 0.79 to 0.81 per cent (0.13 to 0.14 N). The *variation* in neutral salt content in the serum and tissue fluids is probably not enough to materially affect the pH of the fluids through change in the salt effect upon dissociation. Variation in the content of the proteins alters the buffer content of the blood but not significantly the various dissociation constants.

However, changes in total salt and protein concentration in serum and cell fluid cause shifts in the distribution of the ions, through the change in water content, in osmotic pressure and in the Donnan equilibrium.

The Donnan theory of membrane equilibrium states that when two solutions a and b, separated by a semi-permeable membrane, contain both diffusible and non diffusible ions, the diffusible ions distribute themselves thus:

$$\frac{[A]a}{[A]b} = \frac{[A']a}{[A']b} = \frac{[B]b}{[B]a} = \frac{[B']b}{[B']a} \qquad (10)$$

Where A and A′ represent any two species of univalent anions and B and B′ any two species of univalent cations. The brackets here indicate the thermodynamic activities of these ions, the ratios of which are related to but not identical with the ratios of their concentrations, a subject which will not be further discussed in this review. Applied to the serum cell system we have:

$$\frac{[Cl^-]s}{[Cl^-]c} = \frac{[HCO^-_3]s}{[HCO^-_3]c} = \frac{[H^+]c}{[H^+]s} \qquad (10a)$$

The combined effect of water shift and Donnan equilibrium has been discussed by Warburg (1922) and by Barcroft *et al.* (1922). More recently Van Slyke, Wu and McLean (1923) and Henderson, Bock, Field and Adair (1924) have added more data and discussed extensively the question of electrolyte equilibrium as applied to blood.

Relation between pH of blood and serum

Blood is a heterogenous system of serum and cell phases. Each of these phases, to be strictly accurate (Warburg, 1922; Van Slyke, Wu and McLean, 1923) may be subdivided into protein and water phases, but for this review we will consider blood only as divided into serum and cell phases.

Parsons (1917) pointed out that all pH measurements made on whole unhemolyzed blood were in reality measurements of pH of serum, and that all the values reported in the literature determined by electrometric methods on blood represented the pH of the serum of the reduced blood. He further showed in association with Barcroft and others (1922) that the pH of the serum of completely reduced blood is about 0.05 pH less acid than the serum of the blood completely oxygenated at the same CO_2 tension. This indicates a serum pH difference between ordinary venous and arterial blood of about 0.02 pH at the same pCO_2.

Whenever the term "pH of blood" is used it must be understood to mean the pH of the blood serum.

pH of blood cell

The blood cells are more acid than is the serum (Warburg, 1922; Van Slyke, Wu and McLean, 1923; Henderson *et al.*, 1924) by from 0.08 to 0.14 pH at normal serum pH.

Little is known of the actual change in reaction of the blood cells but it exerts an influence on electrolyte and water distribution between cells and serum. With change in anion (Cl^- and HCO^-_3) and H^+ concentration, water is shifted between cells and serum thus changing the cell volume.

Relation between [$BHCO_3$] of blood and serum

The [$BHCO_3$] of serum is higher than that of the cells and therefore higher than the [$BHCO_3$] of whole blood. The determinations of total [CO_2] can be made on either whole blood or serum. The relationship between the [CO_2] of serum and of whole blood is dependent upon the pH of the serum and upon the cell volume. In considering the relation between [$BHCO_3$] of serum and cells it must be remembered

that Na and K ions under ordinary conditions do not diffuse between cells and serum. The shift of base with change in oxygenation and reduction of hemoglobin in the cell results (see equation (8)) in a migration of Cl^-, HCO^-_3 and H^+ through the cell wall and in the serum an exchange of base between Cl^- and HCO_3^-. This changes the relative osmotic concentration in cells and serum and involves a water shift between serum and cells. The relationship between serum and whole blood [$BHCO_3$] has been studied and reviewed recently by Warburg (1922), by Peters and his associates (1923, 1924) and by Van Slyke, McLean and Wu (1923).

Another relationship that is of interest is that between total [CO_2], the value actually determined by CO_2 analysis, and [$BHCO_3$]. The difference between these two quantities is commonly written as [H_2CO_3] in solution and can be calculated if either pCO_2 or pH is known. This relation is discussed later.

At normal pH of 7.3 to 7.4 the [$BHCO_3$] represents about 95 per cent of the total [CO_2].

In studying variation in the acid base equilibrium for clinical and physiological purposes, it is most accurate and convenient to use plasma or serum for both pH and [CO_2] since the influence of any changing cell volume is eliminated.

pH of serum or plasma

For most studies of acid base balance, plasma from 0.3 per cent oxalated blood, and serum may be considered interchangeable, although, as pointed out by Warburg (1922) excessive amounts of oxalate shift the electrolyte between cell and serum and Hooper, Smith, Bell and Whipple (1920) demonstrated significant changes in cell volume arising from dry oxalate used as an anticoagulant. However, plasma is perhaps preferable for colorimetric determinations (see Methods).

Regulation of pH of blood

The manner in which respiration, by increase or decrease of CO_2 tension serves as a mechanism by which the reaction of the blood may be maintained within its normal range, is outlined above in the discussion of the buffer system. The nervous control of this mechanism

is located in a respiratory center of the brain. For many years it was thought that the CO_2 tension was the sole chemical factor that controlled respiration, then with knowledge of the constancy of the pH of the blood, and following the experiments of Hasselbalch and Lundsgaard (1912) it was generally accepted that it is the pH of the blood, which acting upon the respiratory center controls respiration. More recently a different point of view has come to be held (Scott, 1918; Jacobs, 1920; Gesell, 1923; Cullen, Austin *et al.*, 1923; Cullen and Jonas, 1923; Van Slyke, Hastings, Murray and Davies, 1924). The last mentioned workers conclude

"that when there spiratory mechanism is normal increase in alkaline reserve is only partially compensated by increase in CO_2 tension so that increase in pH also occurs. In the same way decrease in alkaline reserve is accompanied by decrease in pH. There is a decrease in CO_2 tension but not sufficient to prevent pH change. The usual percentage change in hydrion concentration is about twice that in CO_2 tension. The arterial CO_2 tension is kept normally between 35 to 45 mm., which is a much narrower range than would be necessitated for the maintenance of normal pH. The conception of the CO_2 tension as a factor physiologically important only from its relationship to blood pH is not consistent with these facts. When conditions force the organism to choose between change in CO_2 tension and change in pH it tends to compromise between the two, and acts in a manner to indicate that maintenance of normal CO_2 tension is in itself an important factor."

Possibly it is the reaction of the respiratory center as distinct from that of the blood, which is the important factor in control. In addition disturbances in the nervous mechanism may change the response to chemical stimulation (J. S. Haldane, 1922). The normal physiological variation in serum between arterial and venous blood during respiration amounts to about 0.04 pH and about 7 mm. of pCO_2 (see charts No. 116 and No. 119 of Henderson *et al.* (1924)).

DETERMINATION OF PH

The methods which have been used in studying pH of blood may be divided into three general groups. The first group includes the electrometric methods which are based upon measurement of an electrometric

potential which is proportional to the H^+ concentration. The "gas chain" or "hydrogen electrode" and the "quinhydron" methods belong to this group.

The second group includes the indirect method of calculating pH either from measurement of buffer ratio or after the method of Barcroft (1914) from change in the constant of Hill's equation for the equilibrium between oxygenated and reduced hemoglobin and the oxygen tension.

The third group comprises the colorimetric or indicator methods which utilize indicators whose solutions give color effects dependent upon the pH.

The principle of these methods are reviewed fully in Clark's (1920) book.

The methods which have been of most value for investigation of blood pH are described briefly.

Electrometric. Hydrogen electrode

The method which has served as the reference method for all pH measurements is the hydrogen electrode or "gas chain" method which is based upon the fact that in a suitable cell the difference of potential between a metal electrode and a solution of its ions is proportional to the ion concentration (or in the newer terminology to the ion activity). Hydrogen gas, when absorbed by a platinum black electrode acts as a hydrogen electrode. This method was used by Hasselbalch and Lundsgaard (1912) in establishing the first exact value of the pH of blood. Parsons (1917) showed that the electrometric determination of pH of reduced whole blood really measures the pH of the serum of reduced blood. Because of technical difficulties due to the presence of oxygen from the hemoglobin it is more accurate and convenient to use serum or plasma than blood. With the technic now available an accuracy of 0.01 pH may be obtained (Donegan and Parsons (1919); Warburg (1922); Cullen (1922)). Although it is the method of ultimate reference, electrometric pH determination requires so much material, time, and experience that ordinarily it is to be used only in physiological studies. It is of interest, however, that Cullen and Biilman find that with proper precautions, the quinhydrone electrode can be used with very small quantities of serum, although it can not be used with whole blood.

Reference standard for pH determination

All hydrogen ion concentrations are referred to a hypothetical "normal hydrogen electrode." It is necessary to base the actual determination upon some reproducible standard of reference. The recent development in the knowledge of electrolytic dissociation and the use of the activity coefficient for hydrogen ion activity has led to some confusion. The reference standard is relatively unimportant in any investigation where only changes of pH are being studied, but becomes of importance when comparing results from different laboratories on such questions as normal pH of blood.

As pointed out later the relation between the pH at different temperatures is also confused in this question of reference standard.

Clark (1920) and Soerensen and Linderstroem-Lang (1924) have agreed that all biological hydrogen ion concentrations shall be reported by them in terms of the older pH values based on conductivity instead of the newer pH activity values.

They also agree that the standard of reference shall be the N/10 calomel cell to which they have assigned definite values at various temperatures. The present authors prefer to standardize their pH determinations against a reproducible standard acid solution either N/10 HCl or N/100 HCl in N/10 KCl, and to assume no change in pH of this standard solution with change in temperature (see Cullen, Keeler and Robinson (1925)).

With the latter standardization, Soerensen's phosphate standards have his values at 18° and are 0.03 pH less at 38°, i.e., a phosphate solution which is 7.40 at 20° is 7.37 at 38°. The pK′ values for serum based on this system and using Bohr's α values are 6.18 at 20° and 6.10 at 38°, giving a temperature difference in agreement with other workers using the N/10 calomel cell standard.

Calculation of pH from $BHCO_3$ content and CO_2 tension

This method using equation 5 requires determination of total CO_2 content, and of CO_2 tension. The total $[CO_2]$ is determined most easily and accurately by Van Slyke's CO_2 apparatus (Van Slyke and Neill, 1924) and the pCO_2 may be determined either by alveolar CO_2 determination (when the subject can coöperate) or from the CO_2 absorption curve. For the latter purpose the total CO_2 content is

determined on one portion of the blood or serum as drawn, and other portions are equilibrated with known CO_2 tensions at two or more points. Analysis of total CO_2 of these equilibrated samples gives data for the CO_2 absorption curve. Peters, Bulger and Eisenman (1924) have recently proposed the construction of the curve from one point (see our equations (16) and (17)). The interesection of this curve with the CO_2 content of the original blood or serum gives the CO_2 tension (pCO_2) as drawn. If venous blood is used it is necessary to correct the total $[CO_2]$ for the change in $[BHCO_3]$ with change in oxygenation of hemoglobin (see equation (18)).

This method, which has been extensively used requires appropriate pK′ values for blood and for plasma or serum. The pK′ values for serum have been recently redetermined for serum, for a variety of clinical conditions (Cullen, Keeler and Robinson, 1925). The value of 6.10 at 38° and 6.18 at 20° are reliable for serum and plasma (see also Warburg (1922) for review of previous work).

The difference between pK'_{serum} and pK'_{blood} used to calculate the pH of serum since the pCO_2 is the same, must be dependent entirely upon the different solubility of CO_2 in whole blood and serum and upon the difference in $[BHCO_3]$. This relation as pointed out above is influenced by cell volume (hemoglobin content) and pH of the serum. This difference, $pK'_B - pK'_s$, has been recently studied by Warburg (1922), by Peters, Bulger and Eisenman (1924), and by Van Slyke, McLean and Wu (1923). The studies including that of Peters, Bulger and Eisenman on a large series of human bloods (see equation (17)) are in close agreement with each other. Hastings (quoted by Van Slyke, Wu and McLean, 1923, p. 800) has represented the relation on a D'Ocagne-Henderson line chart. The values for pK'_s we give in table 1, and for $pK'_B - pK'_s$ in figure o.

The technic which we employ for equilibration to known CO_2 tension and for calculation of data is summarized in a previous paper (Austin *et al.*, 1922).

Colorimetric determination of pH

Because of its simplicity and economy of material and time the direct determination of pH on blood plasma is the method of choice for clinical investigation. The principle of the colorimetric method

involves (1) handling of blood and plasma without loss of CO_2, (2) either elimination of protein error by dialysis or correction for the protein error of the indicator. The colorimetric and electrometric methods applied to protein free salt solutions are in perfect agreement when sufficient precautions are used to prevent loss of CO_2 (Cullen and Hastings, 1922).

The blood may be dialyzed against neutral saline solution and the pH of this dialysate determined by use of an indicator. Levy, Rowntree and Marriott (1915) used this method and introduced the use of phenolsulphonephthalein (phenol red) as indicator. These authors did not prevent loss of CO_2 so that their values did not represent actual pH. Dale and Evans (1920) modified this method by preventing loss of CO_2. Dale and Evans used neutral red and titrated a phosphate control to the same color. Lindhard introduced a micro-modification of Dale's and Evan's method.

Cullen (1922) determined directly the pH of the plasma diluted 1:20 with saline and determined by comparison with electrometric determination the total empirical correction for dilution, salt, protein and temperature. This method has proved convenient for both clinical and physiological studies.

Blood is drawn *without stasis or loss of* CO_2 into a tube under paraffin oil containing oxalate to make 0.3 per cent. It is centrifuged in a stoppered tube completely filled with blood. Especial care is taken that neither the blood or plasma is ever exposed to air. One portion of plasma of from 0.2 to 1 cc. is transferred to 20 volumes of 0.9 per cent NaCl solution containing phenol red, which is already covered with oil. Another portion is added to 0.9 per cent NaCl solution without indicator. The indicator NaCl solution is prepared by adding 1.05 cc. of 0.04 per cent phenol red to 100 cc. NaCl solution and adjusting with N/50 NaOH to pH about 7.5. Phosphate standards at 0.05 pH intervals are used containing 0.01 cc. of 0.04 per cent phenol red per cubic centimeter. The second sample of plasma in saline is used to superimpose the color of the serum upon that of the standard with indicator. The color of the plasma + indicator tube is compared with the combined colors of phosphate + indicator and diluted plasma in a Walpole comparator. For human plasma

$$pH_{38°} = pH_{color} \text{ at } t° + 0.01\ (t° - 20°) - 0.23 \qquad (11)$$

Where "pH_{38}" is the pH of the undiluted plasma at 38°; "t°" is the temperature (15° to 25°) of the phosphate standards and diluted serum when read; "pH_{color}" is the pH at 20° of the phosphate standard which matches the plasma + indicator tube.

Hawkins (1923) has found that the whole blood can be added directly to the saline before centrifuging, thus 0.25 cc. + 5 cc. saline. The empirical correction "C" given in equation 11 as -0.23 varies with different species and is apparently not identical in serum and in plasma and is perhaps more variable in the former. The value for normal human plasma was found by Cullen (1922) to be -0.23 ± 0.04. The extent to which this correction varies under pathological conditions has not however been adequately determined. Not only is the average value for other species different but the individual variation in some species, such as the dog, is apparently greater than in the human. Hastings, Neill, Morgan and Binger (1924) found the average value for twelve pneumonia patients to be -0.27 ± 0.05. Recently Hastings and Sendroy (1924) report that reading both standard and diluted plasma at 38° eliminates this correction. We have found this to be true in some sera but not in all. For the present we must look upon the magnitude of this correction whether the reading be made at 20° or at 38° as open to further investigation. *Calculation of CO_2 tension* may be made directly when total CO_2 and pH determinations have been made.

Summary of equations and constants for expressing relations between certain factors in the acid base equilibrium

It seems desirable for convenience to gather together at this point certain relations between factors concerned in the acid base equilibrium in the blood and used in the calculation of one from another.

The Henderson-Hasselbalch equation for the relation between pH of serum or plasma and CO_2 tension and CO_2 content of the serum or plasma is:

$$pH_s = pK'_s + \log \frac{[BHCO_3]_s}{[H_2CO_3]_s} \tag{12}$$

This may also be written

$$pH_s = pK'_s + \log \frac{[CO_2]_s - \alpha_s pCO_2}{\alpha_s pCO_2} \tag{13}$$

Where for convenience, but contrary to usage, we express CO_2 tension in millimeters, instead of atmospheres so that pCO_2 = mm. CO_2 tension and $\alpha = \frac{1}{760}$ of the value usually given. $[CO_2]_s = [CO_2]$ (at 0°, 760 mm.) of serum or plasma in volumes per cent.

The values for α that have been employed in the past for serum or plasma are based on the observation of Bohr that $\alpha_s = 0.975\ \alpha_{water}$. More recently Van Slyke, Wu and McLean (1923) have found that the ratio of α_s to α_{water} is proportional to the concentration of water in the serum and they have found the average water content of serum to be 91.4 per cent. In table 1, we give both these values for α_s and the corresponding values for pK'_s. In figure 2 we have plotted

TABLE 1

t°	α_{water}	α_S (BOHR)	α_S (V. S. W. M.)
15	0.1340	0.1307	0.1225
20	0.1156	0.1128	0.1057
25	0.0999	0.0975	0.0913
30	0.0875	0.0855	0.0800
38	0.0725	0.0708	0.0663
45	0.0630	0.0615	0.0576
pK'_s at 38°		6.10	6.07

only Bohr's values for α_s since the pK'_s values in that figure are calculated accordingly. The value for pK'_s for human serum or plasma at 38° we take as 6.10 with Bohr's α_s (Cullen, Keeler and Robinson (1925)) or with the lower value for α_s of Van Slyke, Wu and McLean the corresponding pK'_s is lower by 0.028 or in round numbers 6.07.

The Henderson-Hasselbalch equation for the relation between pH of serum and the CO_2 tension and CO_2 content of whole blood is:

$$pH_s = pK'_B + \log \frac{[CO_2]_B - \alpha_B pCO_2}{\alpha_B pCO_2} \qquad (14)$$

Constant values for pK'_B and α_B have commonly been employed in the past differing from pK'_s and α_s by a constant increment. Since, however, these constants for blood differ from those for serum by an increment related to the proportion of cells in the blood, they

can be stated to advantage as a function of the O_2 capacity of the blood.

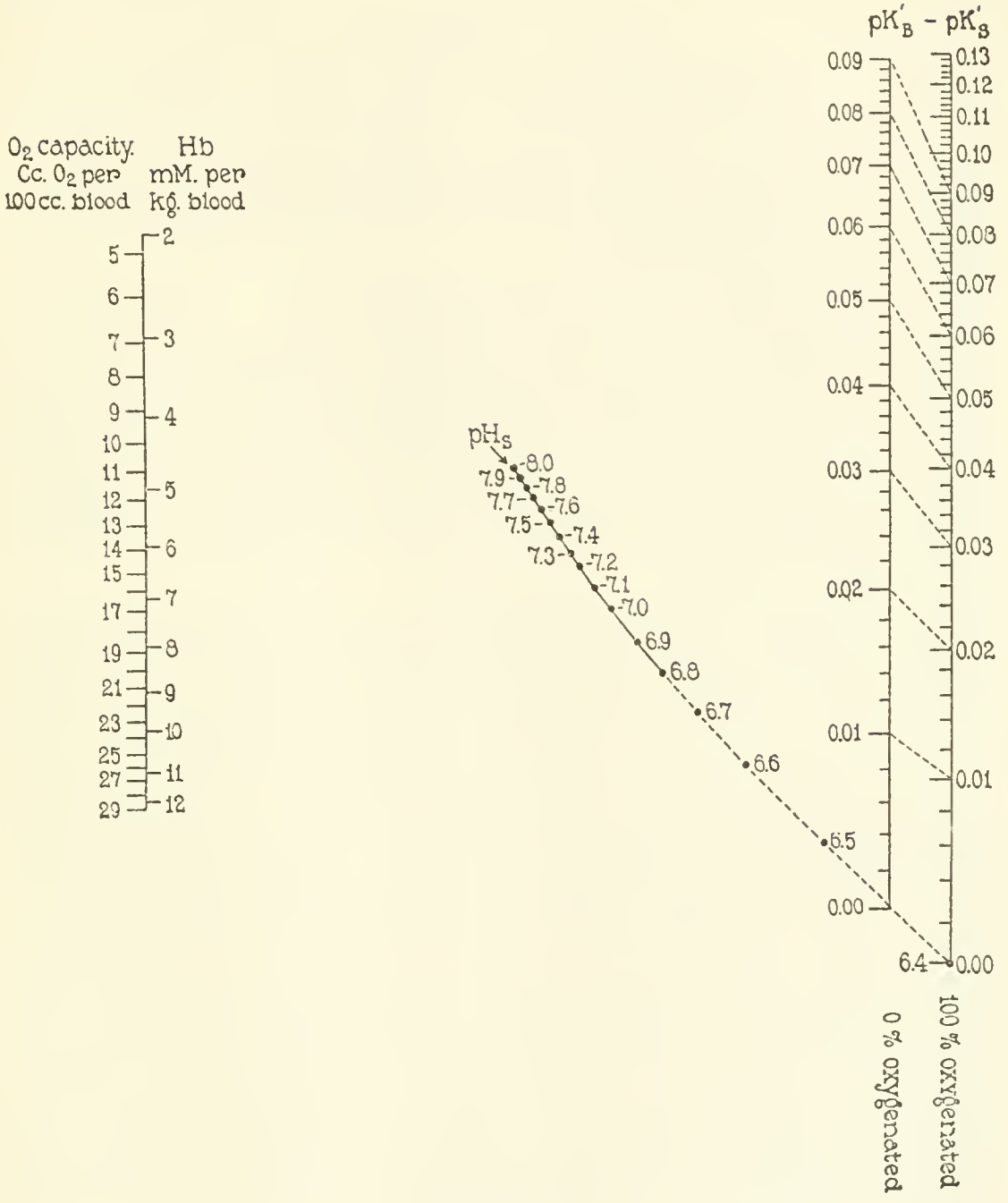


FIG. 0

By the courtesy of Drs. D. D. Van Slyke and A. B. Hastings were are permitted to include this graph prepared by them as a modification of figure 6b (Van Slyke, Wu, McLean, 1923). It permits the reading of $pK'_B - pK'_S$ for any oxygen capacity and pH_S for both fully oxygenated and fully reduced blood with values at intermediate oxygenation by interpolation.

For this purpose we follow Van Slyke, Wu and McLean (1923) using their equation (30). This gives:

$$\alpha_B = (1 - 0.0067\ h)\ \alpha_s \qquad (15)$$

Where h = oxygen capacity in volumes per cent. Using equation (15) to express the relation between α_s and α_B we take as values for $pK'_B - pK'_s$ the graph, figure 6b, from Van Slyke, Wu and McLean (1923). These values they point out agree with the data of Peters, Bulger and Eisenman (1923).

By the courtesy of Drs. D. D. Van Slyke and A. B. Hastings we include a revised form of this graph as figure 0.

The use of CO_2 content and CO_2 tension of whole blood and equation (14) to calculate pH_s is open to much more uncertainty in the values of the constants of the equation than is the use of CO_2 content of serum and equation (13). The latter course is to be preferred wherever possible; this is especially true when blood from different species is under study.

In the study of the carbon dioxide absorption curve of blood we formerly required a knowledge of the CO_2 content of a given sample of blood at three known CO_2 tensions or at three pH values in order to plot the CO_2 absorption curve. Subsequently, however, it was shown by L. J. Henderson (1921) and Warburg (1922) that with changing pCO_2, $[BHCO_3]$ of blood plasma plotted against its pH is approximately linear. Barcroft, Bock, Hill, Parsons, Parsons and Shoji (1922) showed that under the same conditions $[CO_2]$ plotted against $[H^+]$ is approximately linear and Peters, Eisenman and Bulger (1923) showed that under the same conditions log $[CO_2]$ plotted against log pCO_2 is linear. Each of these methods make possible the determination of the CO_2 absorption curve from a knowledge of CO_2 content and either pCO_2 or pH at two CO_2 tensions. Finally for human blood Peters, Bulger and Eisenman (1924) have shown that the slope of log $[CO_2]$ against log pCO_2 can be approximated if the oxygen capacity be known, as follows:

$$\Delta\ [CO_2]_{60-30} = 0.334\ h + 6.3 \qquad (16)$$

where "$\Delta[CO_2]_{60-30}$" is the increase in CO_2 content in volumes per cent between pCO_2 = 30 mm. and pCO_2 = 60 mm., and "h" is the oxygen capacity expressed in volumes per cent. This permits the

approximation of the CO_2 absorption curve for human blood from the knowledge of the CO_2 content at one pCO_2.

The level of true serum absorption curve above the blood curve at 38° has been found by them to be at $pCO_2 = 40$ mm.

$$[CO_2]_s = [CO_2]_B + (0.0159\,[CO_2]_B - 0.281)\,h \qquad (17)$$

Using equations (13), (14), (15) and figure o one can calculate $[CO_2]_s - [CO_2]_B$ for a given blood at any CO_2 tension and the result may be compared at $pCO_2 = 40$ with the value obtained by using equation (17). The calculated values for $[CO_2]_s - [CO_2]_B$ differ considerably, which is probably not surprising when the widely different kind of data on which the various constants are based is considered. The discrepancy emphasizes the need for further experimental study of the relation between blood and serum already pointed out by the authors of these equations in order to define the applicability and limitations of the relations expressed in equations (14), (15), (17) and figure o. The slope of the true serum curve may be approximated from that of the blood curve by assuming that $[CO_2]_s - [CO_2]_B$ is the same at 30 and 60 mm. pCO_2 as at 40, or by assuming that $\log \frac{[BHCO_3]_s}{[H_2CO_3]_s} - \log \frac{[BHCO_3]_B}{[H_2CO_3]_B}$ is 0.01 less at $pCO_3 = 60$ and 0.01 more at $pCO_2 = 30$ than at $pCO_2 = 40$. The result obtained by either method is substantially the same and is approximately consistent with the observed relations. The slope of the true serum curve can also be approximated by equation (16) for whole blood, given above.

When the $[CO_2]_B$ and $[O_2]_B$ of blood as drawn is known and in addition the oxygen capacity and CO_2 absorption curve of the blood, fully oxygenated has been determined at body temperature in vitro and it is desired to determine the CO_2 absorption curve of the blood at the state of oxygen saturation as drawn, we may use the formula of Doisy, Briggs, Eaton and Chambers: at any pH (pH = X):

$$\frac{[CO_2]_B - [CO_2]_{sat.}}{[O_2]_{sat} - [O_2]_B} = 0.44 \qquad (18)$$

where $[O_2]_{sat}$ = oxygen capacity.
$[O_2]_B$ = oxygen content in the state of partial unsaturation.

$[CO_2]_{sat}$ = CO_2 content of blood, at pH = X, when saturated with oxygen.

$[CO_2]_B$ = CO_2 content of blood, at pH = X, when at the oxygen saturation indicated by $[O_2]_B$.

$[CO_2]$ and $[O_2]$ must be expressed in the same units (volumes per cent, mM., etc). The CO_2 absorption curve of the oxygenated blood can then be raised at each pH to a level corresponding to that for the state of unsaturation as drawn and the $[CO_2]_B$ as drawn interpolated on this curve to obtain the pH as drawn, whence the pCO_2 can be calculated.

Logarithmic paper ruled as suggested by Peters (1923) with pH lines is very useful in plotting CO_2 absorption curves. Such a chart can be prepared for serum but not to advantage for whole blood since for its preparation a constant value for α is necessary when pCO_2 is used as abscissae and also a constant value for pK′, but both α_B and pK'_B vary in different bloods according to the oxygen capacity.

Temperature effect

In the biological studies thus far made, interest has centered almost exclusively upon the cause and effect of changes in $[H^+]$ under conditions of constant temperature or where at least change in temperature was not an essential feature of the conditions being investigated. This has been of importance, sometimes unrecognized, in view of certain limitations in the measurements of $[H^+]$ and in view of the relation between $[H^+]$ and $[OH^-]$.

For electrometric measurement of $[H^+]$ at any given temperature a value for $[H^+]$ of some standard solution must as we have already pointed out be more or less arbitrarily assigned for the temperature in question; then at the same temperature the ratio to this of $[H^+]$ of any other solution having been measured, its $[H^+]$ can be calculated with reference to the standard chosen. The various standards in use in this connection we have already discussed.

The direct measurement with the gas chain of an unknown $[H^+]$ at one temperature against a standard $[H^+]$ at another temperature is without any physical significance and cannot be interpreted. For each temperature employed with the gas chain a new value for the $[H^+]$ of the standard solution or cell must therefore be assumed, or calcu-

lated in accordance with some assumption, and no one basis for this assumption has been generally accepted in biological work. The relation of pH = 7.40 at 20° to pH = 7.40 at 38° is one, therefore, that can be stated only when one has defined the standard values employed at each temperature.

The difficulty just stated is one of standardization of measurement. There is, however, a second factor to be considered in dealing with varying temperatures. In aqueous solution $[H^+] \times [OH^-] = K_w$ and at any given temperature, K_w is constant. At constant temperature any increase in acidity always signifies a proportionate decrease in alkalinity. In considering the biological significance of a change of reaction we as a rule make no attempt to distinguish the importance of change in $[H^+]$ and $[OH^-]$ respectively, and at constant temperature such a distinction is of no great significance since they are inversely proportionate.

When we deal with changing temperature the situation alters, however. With change in temperature K_w changes. With change in temperature it would be possible to have simultaneous increase in both $[H^+]$ and $[OH^-]$; change in one no longer implies a reciprocal change in the other. Under these conditions in considering reaction we have three distinct factors we may consider

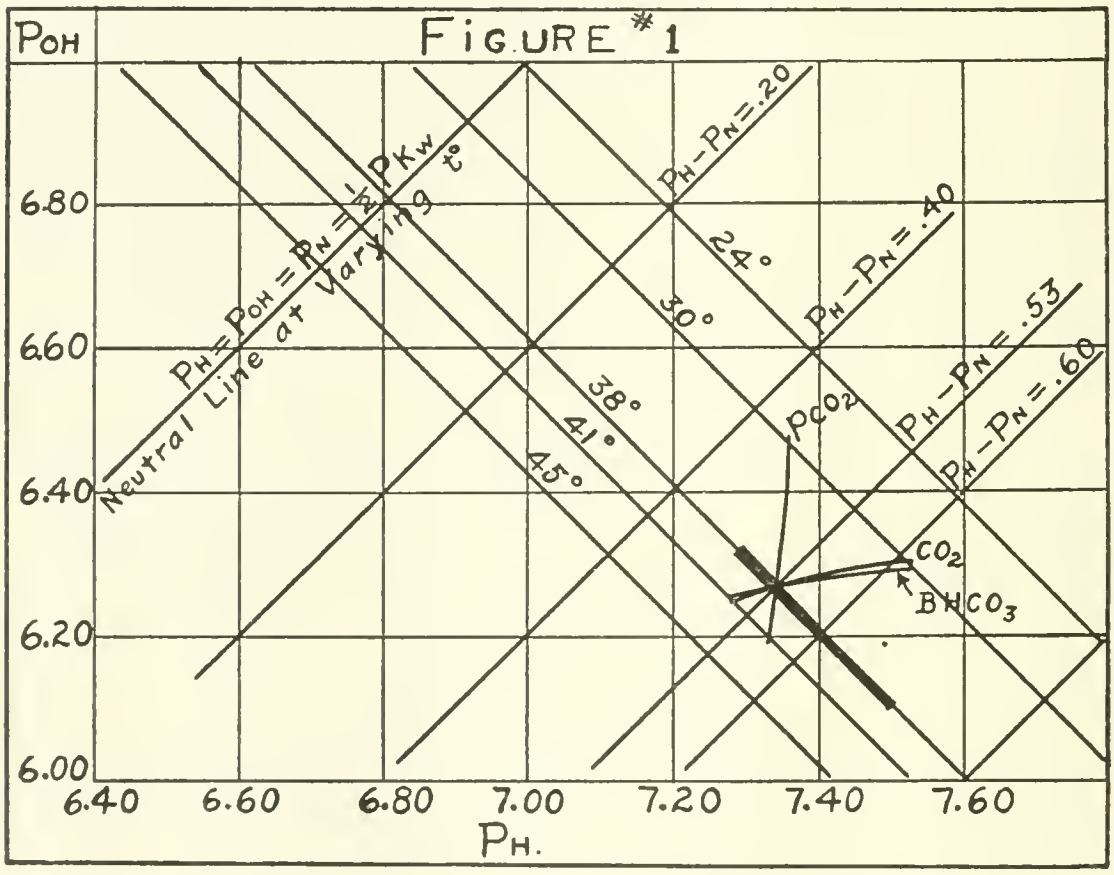
$$(1)\ [H^+],\ (2)\ [OH^-],\ (3)\ \frac{[H^+]}{[OH^-]}.$$

When the ratio of $\frac{[H^+]}{[OH^-]} = 1$, the condition is by definition one of neutrality.

The values for K_w, that is for the product $[H^+] \times [OH^-]$, at different temperatures have been calculated by Lewis and Randall (1923, p. 487) on the basis of Wörmann's determinations of heats of neutralization of strong acids and alkalies at various temperatures. Their values have been used in construction of figure 1, which illustrates the relationships of $[H^+]$ and $[OH^-]$ at varying temperatures. It will be seen from figure 1 that a serum which at 38° has a pH of 7.40 has the same ratio of $\frac{[H^+]}{[OH^-]}$ and a pH equally removed from the neutral point as a serum at 41° with a pH of 7.36.

In biological studies under conditions of varying temperature we

must determine, therefore, whether the significant factor is $[H^+]$ or $[OH^-]$ or the ratio of $\frac{[H^+]}{[OH^-]}$ and our logarithmic notation will be accordingly pH or pOH or 1/2 [pH−pOH], the latter expression measuring the increment of pH to the acid or alkaline side of the neutral point at the temperature in question. Clark (1920) has pointed out that the neutral point although a convenient point of reference is



pH Against pOH at Varying Temperatures

The line of neutrality where pH = pOH at varying temperature.

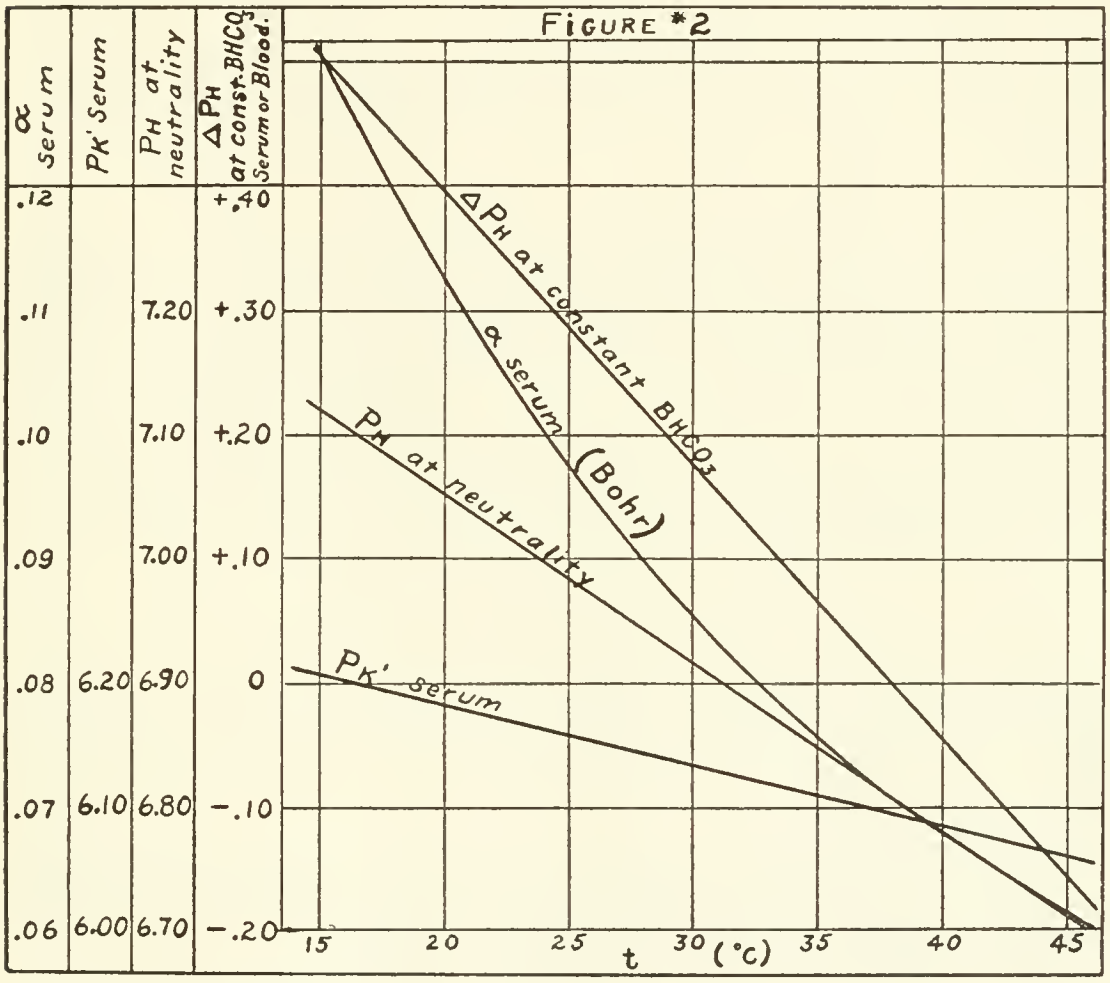
The shaded area is the normal pH, pOH and temperature range of serum.

The curves show the effect of changing the temperature of a particular blood and its true serum at constant pCO_2, at constant $BHCO_3$ and at constant total CO_2.

not as a rule characterized by any striking change in chemical behavior. We may, however, expect to find in some reactions the major importance attaching to pH and in others to pOH.

In the study of blood acid base equilibrium under conditions of varying temperature there are four variables of which the temperature coefficient must be taken into account.

1. The solubility of CO_2. The solubility of CO_2 in serum is given in table 1 and figure 2. So far as we know equation (15) is applicable at any temperature under consideration.



(1) α-serum in equation:

$$[H_2CO_3]_s \text{ (vols. per cent)} = \alpha_s \, pCO_2 \text{ (in millimeters)}$$

using Bohr's data.

(2) PK′ serum in equation:

$$pH = pK'_s + \log \frac{[BHCO_3]_s}{[H_2CO_3]_s} = pK'_s + \log \frac{[CO_2] - \alpha pCO_2}{\alpha pCO_2}$$

using Bohr's α.

(3) pN = pH at neutrality = pOH at neutrality. pOH = 2pN − pH.

(4) Δ pH at constant $[BHCO_3]$; permits construction of CO_2 absorption curve at changed temperature.

2. The value of pK'_s at varying temperatures has been recently determined by Cullen, Keeler and Robinson (1925) and the result of their observations is given in figure 2. The observed change with

temperature is only a little more than half that calculated by L. J. Henderson (1908) and by Stadie and Martin (1924) from the data in the literature upon the heat of ionization of H_2CO_3. The explanation for this discrepancy is not clear; possibly it lies in the choice of values for standardization of pH measurements at varying temperatures. In any case, however, the values shown in figure 2 are those which are consistent with our present methods and standardization in blood equilibrium studies.

3. Change in the location of the CO_2 absorption curve occurs due to different heats of ionization of protein acids and H_2CO_3. Or stated in other words the shift of the CO_2 absorption curve is due to unequal changes in the K_1 and K_3 of equation (4). Experimental data on the magnitude of this change is very scanty. Stadie and Martin have data which indicate that the CO_2 absorption curves of whole blood are approximately parallel at 38° and at 15° and that the change in pH at the same $[BHCO_3]$ is given approximately by the equation

$$\Delta pH_{[BHCO_3]} = 0.02\ (38° - t°) \qquad (19)$$

Austin and Robinson have found this factor to be 0.02 ± 0.003 for both whole blood and true serum in the dog and sheep. Stadie has observed the change for separated serum in the dog and sheep to be within these limits.[1]

The advantage of considering the change in location of the CO_2 absorption curve with change in temperature in terms of change of pH at constant $[BHCO_3]$ may be explained as follows:

There is in a given sample of blood or serum a certain amount of base combined with strong acids (chiefly Cl′). The amount of base bound by these acids is not significantly effected by change in temperature. The remaining base is bound by weak acids, chiefly protein acids and HCO_3', in accordance with equation (4). The values for K_1 and K_3 in this equation vary with temperature and unequally. If as an approximation we neglect the phosphates and assume that the base free to combine with weak acids is given by $[BHCO_3] + [BPr]$ it follows that when under conditions of changing temperature we keep $[BHCO_3]$ of a sample of blood or serum constant we will also keep [BPr] constant. Since $[BPr] + [HPr]$ is constant in a given sample

[1] Unpublished data from this laboratory.

we consequently keep the ratio $\frac{[HPr]}{[BPr]}$ constant. Under these conditions change in H^+ is related to change in K_3 with change in temperature as follows:

$$\frac{[H^+]_t}{[H^+]_{t'}} = \frac{K_{3t}}{K_{3t'}}$$

or in the logarithmic form

$$pH_t - pH_{t'} = pK'_{3t} - pK'_{3t'}$$

Change in pH with temperature at constant $[BHCO_3]$ is therefore a measure of the change of pK′ of protein with temperature. It is reasonable to expect a reasonable constancy in the change of pK′ of blood proteins with temperature and hence of pH at constant $[BHCO_3]$ as indicated in equation (19).

Accepting the value of 0.02 for $\Delta\, pH_{[BHCO_3]}$ tentatively for the present we indicate in figure 2 its magnitude at varying temperatures. It must be pointed out that if the pH at constant $[BHCO_3]$ with varying temperature is approximately the same for separated serum, true serum and whole blood, the $\Delta\, [BHCO_3]$ at constant pH with varying temperature will be much less for separated serum than for true serum or for whole blood due to the difference in the slope of their respective buffer curves.

The change in $[BHCO_3]$ with change in temperature at constant pCO_2 or at constant $[H_2CO_3]$ can be readily calculated from equation 19 and from the CO_2 absorption curve if the slope of the latter is known or can be approximated.

This relationship is shown in table 2.

4. At constant temperature $\Delta pH = -\Delta pOH = -\Delta(pN-pH)$ where pN = pH at neutrality = pOH at neutrality = 1/2 pK water. The variation of pN with temperature (from pK_w calculated by G. N. Lewis) is shown in figure 2. At any temperature pOH = 2 pN − pH. Therefore with change in temperature between 15° and 45° there is at constant pH the following change for each rise of 1°C.

$$\Delta\,(pN - pH) = -0.0143.$$

$$\Delta\, pOH = -0.0286$$

Taking these four changes into account the relations for a particular true serum at 30°, 38° and 41° are shown in figures 1 and 3. The CO_2

absorption curves in figure 3 represent the approximate calculated change in the true serum from the blood of W. C. S. from the data of Stadie and Martin (1924). It will be seen that when the blood temperature is increased from 38° to 41° and the CO_2 tension is kept constant the following ensue:

Slight reduction of pH (slight increase of $[H^+]$)

Marked reduction of pOH (marked increase of $[OH^-]$)

Marked decrease in the ratio $\frac{[H^+]}{[OH^-]}$

Decreased CO_2 capacity.

TABLE 2

Effect of change in temperature on pH of true serum and separated serum various factors being kept constant

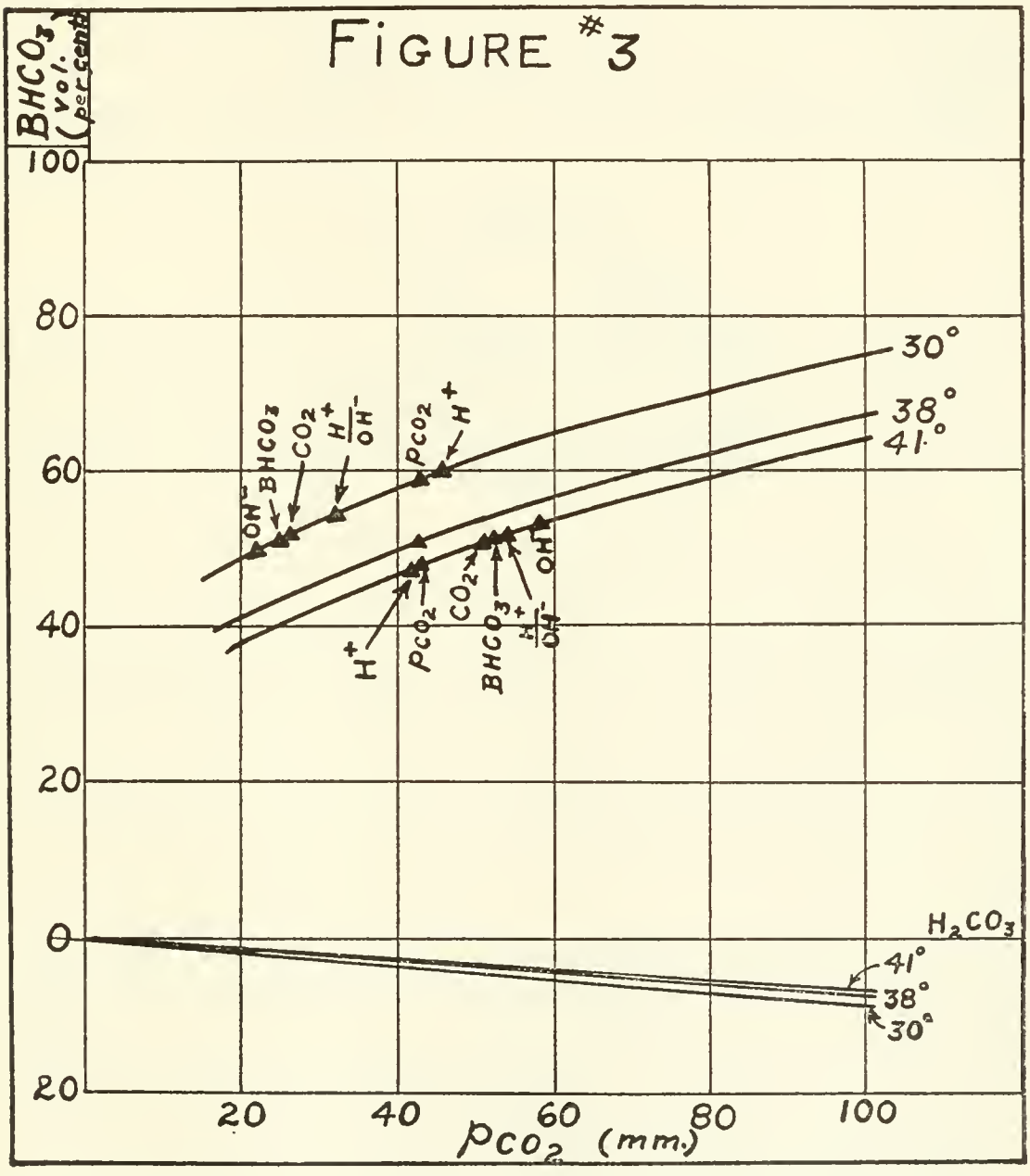
(Calculated from equations (19) and (5))

TEMPERATURE	ASSUMED CONDITION	$[CO_2]$	pCO_2	$[H_2CO_3]$	$[BHCO_3]$	pK′	pH	ΔpH	log R
True serum $\frac{d[BHCO_3]}{dpH} = -62$ (t° constant)									
		vols. per cent	*mm.*	*vols. per cent*	*vols. per cent*				
38°	Initial	54.1	43.3	3.07	51.0	6.100	7.320		1.220
41°	Constant $[BHCO_3]$	54.4	51.2	3.41	51.0	6.085	7.260	−0.060	1.175
41°	Constant pCO_2	51.0	43.3	2.88	48.1	6.085	7.308	−0.012	1.223
41°	Constant $[H_2CO_3]$	52.3	46.0	3.07	49.2	6.085	7.290	−0.030	1.205
Separated serum $\frac{d[BHCO_3]}{dpH} = -14$ (t° constant)									
38°	Initial	54.1	43.3	3.07	51.0	6.100	7.320		1.220
41°	Constant $[BHCO_3]$	54.4	51.2	3.41	51.0	6.085	7.260	−0.060	1.175
41°	Constant pCO_2	53.0	43.3	2.88	50.1	6.085	7.325	+0.005	1.240
41°	Constant $[H_2CO_3]$	53.5	46.0	3.07	50.4	6.085	7.300	−0.020	1.215

Under these conditions fall in pH (increase in $[H^+]$) does not mean diminished alkalinity.

These relations are also shown in figure 1. It will be seen that change in temperature at constant pCO_2 causes little change in pH but marked change in pOH. On the other hand change in tem-

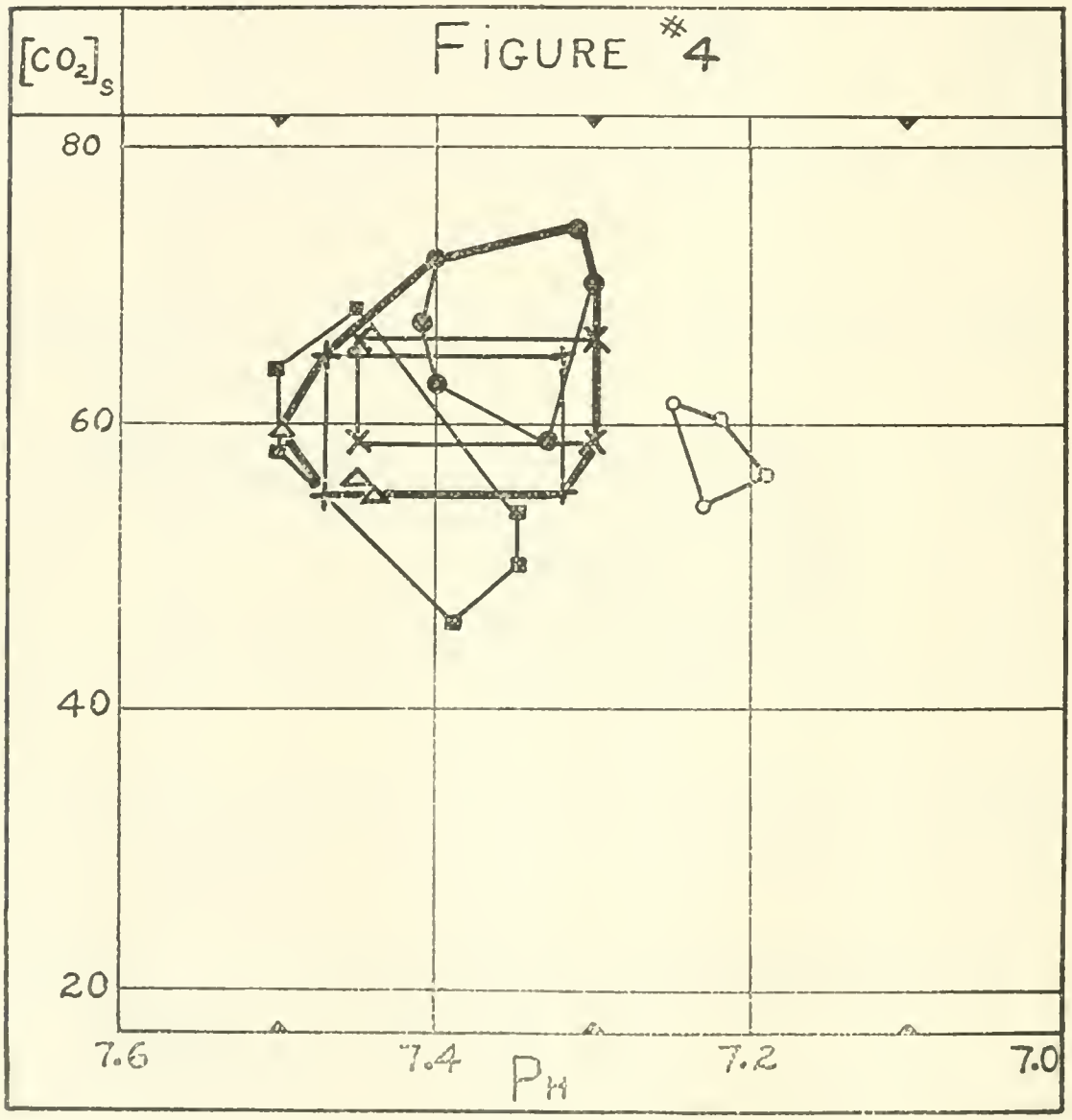
perature at constant [CO_2] or [$BHCO_3$] causes marked change in pH and little change in pOH. These data permit us to apply our methods of calculating to the case of febrile blood or to blood from chilled or heated extremities.



THE CO_2 ABSORPTION CURVES FOR TRUE SERUM OF THE SAME BLOOD AT THREE TEMPERATURES ARE SHOWN

An initial point is indicated on the 38° curve and on the other curves are indicated the points which have the same pH, pOH, $p\frac{(H^+)}{(OH^-)}$, [$BHCO_3$], [CO_2] and pCO_2 as the initial point.

When blood is obtained at a temperature other than 38° and its $[CO_2]$ determined and in addition its CO_2 absorption curve at 38° is determined the following corrections must be introduced to determine pCO_2 and pH as drawn:



NORMAL PLASMA $[CO_2]$ AND pH VALUES

•———• Cullen and Robinson (1923).

x———x Marrack and Boone (1923).

+———+ Myers and Booher (1924).

Δ Kochler (1923).

▩——— ▩ Hastings, Neill, Morgan and Binger (1924); $[CO_2]s$ values raised from blood to serum values but not corrected from arterial to venous values.

°———° Chambers and Kleinschmidt (1923).

▬▬ Values taken as normal for purposes of this review.

1. Relocation of curve for unsaturation (equation (18))
2. Relocation of curve for temperature (equation 19 or Δ pH at constant $BHCO_3$ from figure 2)
3. Use of α_s pK'_s and pN for appropriate temperature (see figure 2).

When blood is obtained at a temperature other than 38° and its $[CO_2]$ determined and its pH at 38°, the correction to original pH is given by equation 19. This equation is at present an approximation only and can therefore be used for constant $[CO_2]$ as well as for constant $[BHCO_3]$.

NORMAL pH VALUES

pH of normal serum and plasma

The range of normal variation in serum pH was given by Van Slyke (1921b) as pH 7.3 to 7.5. Cullen and Robinson (1923) studying normal students with the colorimetric method found a variation from 7.28 to 7.41. All but two, 7.28 and 7.41 fell within the limits 7.40 to 7.30.

Bigwood (1923) with another series with the same method confirms this variation of 7.30 to 7.40. Myers and Booher (1924), using the same dilution but reading the determination in Myers colorimeter found the limits of normal pH to lie between 7.35 and 7.43. However, Koehler (1923) and Marrack and Boone (1923) found somewhat higher normal pH values as do Hastings, Neill, Morgan and Binger (1924) in arterial blood. The values of Chambers and Kleinschmidt (1923) are much lower and must represent a different pH standardization. We have taken for this review the normal outline indicated in figure 4 with pH range from 7.30 to 7.50.

In the observations of Cullen and Robinson the zone 7.30 to 7.40 was found normal not only for various individuals but for a single individual. That is, the pH of any individual might on successive days vary between 7.30 and 7.40.

Drucker and Cullen have developed a technique for taking capillary blood from the heel of infants or the ear of adults without loss of CO_2, using Hawkin's modification of Cullen's method for pH and employing 0.25 cc. of blood. They find the pH of normal infants' capillary blood to lie between 7.34 and 7.44. Since the capillary blood has been shown by Lundsgaard to approximate arterial blood in

composition its pH will be about 0.03 more alkaline than venous blood. The range observed, therefore, is similar to that found by Cullen and Robinson for adults.

pH of body fluids other than serum

As stated by Van Slyke (1921b) our limited knowledge of body fluids indicates that extracellular fluids have approximately the same pH as serum. Parsons and Shearer (1920) report that the pH of cerebrospinal fluid is that of normal plasma pH. Boots and Cullen (1922) found in nonpurulent joint exudate in rheumatic fever pH values of 7.3 to 7.4. In this laboratory we observed the same pH for edema fluid and blood serum.

pH of purulent fluids. Whenever the pH of purulent fluids has been measured it has been found more acid than the serum. In purulent joint exudates Boots and Cullen (1922) found pH of 6.40. Lord has reported pH of 6.8 to 6.2 in consolidated portions of lungs in lobar pneumonia. This range has also been observed by Avery and Cullen (unpublished).

Intracellular pH. Little is known of intracellular reaction other than that of the red cells which is estimated by Warburg (1922) and by Van Slyke, Wu and McLean (1923) for normal serum pH of 7.4 to be 0.08 to 0.14 pH more acid than serum. Probably other intracellular fluids vary, at least as much from that of serum.

pH of secretions. It is interesting that the cells of the body although bathed with fluid of such constancy of pH as serum, can function in contact with a very high degree of acidity. The pH of gastric juice may be less than 2. The pH of urine ranges from 5.0 to 8.5; duodenal fluid may have a pH over 8.0. Saliva has a normal pH of 6.7 (Starr, 1922).

pH OF PLASMA IN DISEASE

Abnormal acid base balance

The terms acidosis and alkalosis, common in clinical usage, are often used by different individuals to describe entirely different conditions. Because the first recognition of abnormal acid base balance came through the knowledge that in diabetes abnormal metabolism resulted in the excretion of aceto-acetic acid, β-oxybutyric acid and acetone,

the term acidosis is often used to describe either a ketonuria or a ketonemia. The term "acidosis" has been applied to the following conditions, (1) acetone bodies in the urine (2) acetone bodies in the blood, (3) decreased alkali reserve, (4) decreased CO_2 tension of alveolar air, (5) and decreased pH of plasma.

This confusion is well recognized in physiological work and various attempts have been made to establish a more precise terminology. Van Slyke (1921b) in his review of abnormal and normal acid base balance divided the possible conditions of abnormality into six groups which are best visualized from his diagram which is used with slight relocation of the normal area as the basis of figure 5.

The Report on Acidosis of the British National Research Council, proposes the terms alkalosis and acidosis to indicate high and low levels of $[BHCO_3]$ and alkalemia and acidemia to indicate high and low pH. Hasselbalch, Haldane, Van Slyke and the present authors (in studying the acidosis of anesthesia) have used "true acidosis" to mean coincident abnormally low pH and $[BHCO_3]$.

It is probable, however, that no general usage for the term acidosis can be agreed upon and it is therefore better to avoid the term wherever possible or else to state the exact sense in which it is used.

For the present it is best to state the conditions of the acid base balance in terms of two of its factors; either in terms of Van Slyke's nine areas, or perhaps simply as an acid base condition of 7.4 and 50, indicating $pH_s = 7.4$ and $[CO_2]_s = 50$ volumes per cent. The term "A.B.C. (acid-base condition of 7.4 and 50)" might supply the need for an easily handled term. The nature of the coördinates 7.4 and 50 make confusion between them impossible. Whatever the terminology, it is evident that determination of only one of the three variables pCO_2, pH or $[CO_2]$ is not sufficient to determine the acid base condition.

The studies of the alkali reserve of the blood in disease are much more numerous than those dealing with direct measurement of the pH of the plasma. Simultaneous studies of CO_2 tension and CO_2 content of the plasma in disease from which the pH can be calculated are also rare.

Accumulation of acids other than HCO_3^- in the blood with depletion of the blood bicarbonate has been described as a rule as occurring at

first without change in pH and only after considerable reduction in the bicarbonate to be associated with a more acid serum pH. Such a course of events is approached in the acidosis of fasting, diabetes and nephritis in figure 5. The compensated stage of alkali deficit was believed to be brought about through a response of the respiratory center to an unmeasurably small diminution in pH with consequent stimulation of pulmonary ventilation, and consequent diminution in pCO_2 in alveolar air and arterial blood just sufficient to secure an almost proportionate reduction of bicarbonate and of CO_2 tension with almost constant pH.

Only when the respiratory mechanism fails to function adequately and to compensate was change in pH believed to occur. With the introduction of Cullen's (1922) method for direct measurement of the pH of serum or plasma as drawn and the application of this method to disease we are recognizing, however, that fall in plasma bicarbonate due to introduction of other acids, is often associated throughout its course with rise and fall of pH as indicated by the numbered arrows in figure 5. Evidence for this is to be found in the studies we describe of acid and alkali administration, of anesthesia, of exercise and radiation. This behavior of blood reaction has a bearing upon the studies of the regulation of the respiratory center and the relative importance of pH and of pCO_2 in this regulation. This has been previously discussed.

Excessive introduction of acids

Many experimental studies have been made upon the effects of acid fed by mouth or administered intravenously. The effect, we have observed in the dog, from injecting HCl, H_3PO_4, NaH_2PO_4 and lactic acid is shown in table 3. These injections were made under local anesthesia into the vein (in one case into the heart) using M HCl, M/10 H_3PO_4, M/10 NaH_2PO_4 and M/1 lactic acid.

The change in pH and $[BHCO_3]$ is shown in table 3. The direction and magnitude of the change in the acid base equilibrium is shown by the numbered arrows in figure 5.

The apparently less marked effect of H_3PO_4 on the $[BHCO_3]$ and its greater effect on pH is due to the large amount of acid injected leading to almost complete disappearance of $BHCO_3$ from the blood. The

less pronounced effect of NaH_2PO_4 both on bicarbonate and pH is to be expected. The absence of effect of lactic acid on pH is, however, interesting. At the pH of blood the ratio of $\frac{\text{lactic acid}}{\text{sodium lactate}}$ is about $\frac{1}{3200}$, taking the dissociation constant of lactic acid as 1.38×10^{-4} (Landolt-Börnstein 4th edition p. 1147). The markedly diminished effect of lactic acid per millimole on the bicarbonate as compared with HCl is therefore not to be expected except as a result of disappearance, of the lactic acid, as for instance, by synthesis to glucose or as a result of its wider distribution in the body fluids as in the case of bicarbonate, later described.

TABLE 3

ACID	CONCENTRATION	AMOUNT INJECTED PER KILO	TIME OF INJECTION	$\Delta[BHCO_3]$ PER MILLIMOLE OF ACID PER KILO	$\Delta[pH]$ PER MILLIMOLE OF ACID PER KILO
	M	*mM.*	*minutes*	*mM.*	
HCl	1.0	2.2	10	−6.0	−0.11
HCl	1.0	2.2	10	−7.0	−0.06
HCl	1.0	1.4	6	−6.0	−0.06
H_3PO_4	0.1	4.9	49	−4.0	−0.16
NaH_2PO_4	0.1	4.9	52	−2.0	−0.03
Lactic	1.0	2.2	6	−2.0	+0.01

When HCl is given by mouth the change in plasma bicarbonate and pH depends on the relative rate of absorption and of compensating excretion. In the observation of Gamble and Ross (1923) the administration to a child of 17 mM. per kilo of HCl in 2 days lowered the plasma bicarbonate 9 mM. and the pH 0.17. Haldane (1923) and Gamble and Ross (1923) pointed out that the ingestion of ammonium chloride in man causes marked and prolonged acidosis, due presumably to conversion of part of the ammonia into urea, thus freeing the acid which had been combined with it. The acidosis is shown by fall in the plasma CO_2 capacity and fall in the alveolar pCO_2. There is a roughly commensurate increase in the rate of acid plus ammonia excretion in the urine, the ratio between the increase in acid and increase in ammonia being dependent upon the phosphates available for excretion. As acidosis continues the phosphate excretion falls,

and at this time a glucose tolerance test produces greater rises in blood sugar. The fall in plasma bicarbonate is replaced almost molecule for molecule by chloride in Haldane's studies.

$CaCl_2$ ingested causes similar change in the plasma bicarbonate and chlorides as shown by Gamble, Ross and Tisdall (1923). The Ca^{++} is mainly excreted as $CaCO_3$ in the feces and the Cl^- is absorbed, replacing HCO_3^- and causing true acidosis with low pH and low $[BHCO_3]$.

Both NH_4Cl and $CaCl_2$ are powerful diuretics. Haldane produced a fall in weight of 7 pounds in three days after 65 grams of NH_4Cl and a rise in hemoglobin of 20 per cent even although he drank water freely; and Levy noted a similar increase in serum protein after $CaCl_2$ ingestion. During NH_4Cl ingestion Haldane observed the following urinary excretions expressed in percentage of the normal values: Na 250 per cent, K 520 per cent, Ca 330 per cent, P 180 per cent. The diuresis and the salt excretion are attributed by Haldane to the fact that the colloids of the body, being brought nearer their isoelectric point by the fall in pH retain less water and base.

The serum Ca has been found by Haldane (1924) to increase 10 per cent following NH_4Cl ingestion. $CaCl_2$ ingestion can cause an increase of 25 per cent in serum Ca. Sodium bicarbonate conversely lowers the serum Ca 10 to 20 per cent. Ammonium chloride has been used in the treatment of tetany by Freudenberg and Gyorgy and in lead poisoning by Aub and his associates with striking effect. In infantile tetany the symptoms vanish in a few hours and satisfactory results have also been obtained in gastric tetany and post operative tetany. In a series of infants treated with 0.5 gram per kilo per day of ammonium chloride the serum calcium rose from a mean value of 6.6 mgm. per 100 cc. to 8.9, the phosphorus falling from 4.9 mgm. per 100 cc. to 2.9. Gollwitzer–Meier (1924) has reported a fall in plasma bicarbonate after intravenous ingestion of $MgCl_2$ in rabbits.

Excessive introduction of base

Introduction of base into the body increases the bicarbonate of the blood and the pH. Oral bicarbonate administration in human beings has been found by Palmer and Van Slyke (1917) to raise the bicarbonate of the blood according to the assumption that the body contains

70 per cent of fluid and the bicarbonate is distributed throughout this fluid. According to this assumption the following equation was calculated. Increase in plasma CO_2 in volumes per cent $= \frac{38g}{W}$, g being grams of sodium bicarbonate administered and W the body weight in kilos. Or this may be stated that for each mM of bicarbonate administered per kilo, the increment in serum bicarbonate is 1.43 mM. This indicates a much wider distribution of the orally administered bicarbonate than of injected HCl or H_3PO_4 in our experiments described above. In our experiments with intravenously injected HCl the effect is as if distributed in 1/6 or 1/7 of the body weight instead of in 70 per cent of the body weight. The direction of the change in the acid base equilibrium following alkali administration is shown in figure 5.

Excessive loss of acid from body

Loss of HCl from the body such as occurs in pyloric obstruction also gives rise to increase in plasma bicarbonate (MacCallum *et al.* 1920; Hastings, Murray and Murray, 1921; Grant, 1922), although in the experimental studies of Hastings, Murray and Murray the rise in bicarbonate was not associated with any significant rise in pH.

Renal disease

Since the elimination of acid from the body is chiefly by CO_2 elimination through the lungs and by excretion of other acids as acid phosphates and combined with ammonia through the kidneys, it might be expected that impaired renal function would induce acidosis. Nephritis and other forms of renal disease associated with impairment of renal function do as a matter of fact give rise to disturbance of acid base equilibrium. Wallace and Pellini (1921) point out, however, that double nephrectomy does not produce acidosis in animals and therefore attribute the acidosis of nephritis to some other factor than mere impairment of acid excretion. Lepine (1879) and von Jaksch (1888) by titration of the ash of blood demonstrated a diminished alkalinity in the blood of uremics. Straub and Schlayer (1912) measured the CO_2 tension of the alveolar air in eight cases of uremia and found in the patient with highest values 34 to 39 mm. pCO_2 and in the patient with the lowest, 11 to 17 mm. On the basis of this and

the hyperpnea of uremics they suggested that acid intoxication is a factor in uremia. Von Hoesslin (1912) pointed out the influence of alkali administration in diminishing the albuminuria and cylindruria in some cases of nephritis and called attention to the difference in amount of alkali necessary to change the reaction of the urine in different cases. Sellards (1912) introduced the measurement of the amount of $NaHCO_3$ necessary by mouth to render the urine alkaline as a test for the detection of acidosis and found that whereas 5 grams by mouth suffices in the normal individual, this dose was effective in only one out of nine patients with chronic diffuse nephritis and two patients tolerated 60 and 130 grams respectively intravenously and still excreted acid urine. Palmer and Van Slyke (1917) showed that the depletion of the alkali reserve could be better gauged by noting the amount of alkali that must be administered to cause excretion of a *less acid* urine rather than of an *alkaline* urine.

Peabody (1914, 1915) showed that as chronic diffuse nephritis increases in severity as measured by impairment of phthalein excretion and retention of non-protein nitrogen in the blood there appears at first little or no evidence of acidosis, then an increase in Sellard's tolerance to alkali and finally in advanced cases, verging on uremia, a diminution in the alveolar CO_2 tension which in the terminal stages of uremia may be very marked. He noted there was not a strict parallelism between the other evidences of impairment in renal function and the degree of acidosis but only a general tendency for both to become marked in advanced cases. The dyspnea of cardiorenal disease also was commonly more marked than could be accounted for merely by the acidosis. Lewis and his coworkers (1913), and Palmer and Henderson (1913) came to substantially these conclusions. Chase and Myers (1920) using the method of Van Slyke and Cullen (1917) for the CO_2 capacity of the venous plasma similarly demonstrated this acidosis. Marriott and Howland (1916) demonstrated an increase in the phosphate of the serum in nephritis with lowered CO_2 capacity of the plasma and with decreased plasma pH by the dialysis method of Levy, Rowntree and Marriott. Greenwald (1915), Feigl (1917a, 1917b), Denis and Minot (1920), Salvesen and Linder (1913) have also shown retention of acid phosphate in nephritics. Means and Rogers (1917) reported an extreme acidosis in a man with bilateral polycystic kid-

neys complicated by a septic infection of the hand. Two days before death he had extreme hyperpnea with a ventilation of 51 liters per minute, an alveolar pCO_3 (Plesch) of 6.4 mm., a blood urea of 332 mgm. per 100 cc. (55 mM.), a CO_2 capacity of the plasma of 12 vols. per cent (5.4 mM.), no phthalein excretion in three hours, serum phosphorus of 18 mgm. per 100 cc. (5.8 mM.) serum calcium 3 mgm. per 100 cc. (0.75 mM.). At this time 110 grams $NaHCO_3$ by mouth failed to render the urine alkaline.

If one compares the retention of phosphate with the diminution of bicarbonate in the serum it would seem that the acidosis can not always be explained simply by the phosphate retention however. In Means and Rogers case with a rise of [P] above the upper normal of 4.3 mM. representing about 8 meq. of base, there is a fall of $[BHCO_3]$ below the lower normal of 13 mM. Some other factor appears to be concerned here than merely phosphate retention. The data of Salvesen and Linder (1923) show very little change in the serum base in nephritics beyond the normal limits which makes it probable that acid other than HCO_3^- is increased.

MacNider (1920) has demonstrated disturbances in the acid base equilibrium in experimental nephritis in animals and here the acidosis is possibly even more conspicuous than in clinical nephritis. This author and also Nagayama (1920) and others have demonstrated improvement in renal function when the disturbance in acid base equilibrium is corrected by alkali therapy and MacNider has demonstrated a diminished susceptibility of the kidney to injury by toxic substances when by alkali therapy the acidosis is prevented.

While these studies indicate the depletion of alkali reserve in nephritis, direct studies of the pH of the blood as it exists in vivo such as are furnished by the method of Cullen (1922) are at present available in the literature for only a few cases. Cullen and Jonas (1923) cite one case of uremia with plasma pH 6.7 and $[CO_2]$ of 4 vols. per cent. Cullen and Stillman at the Rockefeller Institute found a pH of 7.12 with a low normal $[CO_2]$ of 50 vols. per cent in the plasma of a patient with acute convulsions and unconscious but without uremia. Strenuous alkali therapy brought the pH to 7.35 and the $[CO_2]$ to a high normal and the patient recovered from the attack. This case is a striking example of the value of pH studies. Linder,

Hiller and Van Slyke (1925) report 17 observations on 10 patients with various types of nephritis. One case of nephrosclerosis and one of chronic nephrosis showed high normal bicarbonate with normal pH. In those forms of glomerulonephritis associated with marked retention of urea there is a tendency to depression of both bicarbonate and pH; the lowest observation being [$BHCO_2$] = 27.8 volumes per cent with pH = 7.16. The zone occupied by these cases is shown in figure 5. Myers and Booher (1924) report 15 cases of nephritis among their 64 cases of abnormal acid base balance, seven of these with less marked depression of [CO_2] showed pH within the lower limit of normal (Hasselbalch's and Van Slyke's compensated acidosis) while eight cases showed abnormally low pH and more marked depression of [CO_2] (uncompensated or true acidosis). However, if the observations on the 15 cases be plotted as in figure 5, the distinction between the compensated and uncompensated groups is seen to be largely an artificial one, due to the width of the normal pH zone. The evidence is that any lowering of [CO_2] tends to be associated with more acid pH.

Cardiac disease

In cardiac disease uncomplicated by impairment of renal function Peabody (1914) found no evidence of alteration of pH nor of acidosis that could account for the hyperpnea. Wilson, Levine and Edgar (1919) found normal CO_2 capacity of the plasma in cases of "irritable heart."

Diabetes mellitus

Diabetes mellitus because of the accumulation of abnormal products of metabolism has long been the classic example of disturbance of acid base balance. Of the older literature in regard to presence of acetone bodies there are many reviews. The relation of alkali reserve to alveolar CO_2 and to titratable alkali was shown by Stillman, Van Slyke, Cullen and Fitz (1917). It has long been recognized that low alveolar CO_2 tension is an index of low alkali reserve. If the low [$BHCO_3$] is accompanied by sufficient decrease in pCO_2 to prevent change in pH, the condition is that of "compensated acidosis." (Hasselbalch; Area 6, Van Slyke). It was also recognized that coma of diabetes is associated with a true acidosis in the sense of decreased pH and decreased [$BHCO_3$].

Before the discovery of insulin diabetic coma was only occasionally strikingly benefitted by alkali treatment.

One of the most impressive demonstrations of the action of insulin is afforded by the changes in the acid base balance in diabetic acidosis following insulin treatment. Chart No. 3, p. 547, Cullen and Jonas (1923) shows this most clearly. A patient with a plasma pH of 6.98 and [$BHCO_3$] of 16 vols. per cent had his plasma restored in one day to a normal range with a plasma pH of 7.32 and [$BHCO_3$] of 41.5 vols. per cent. The insulin altered the metabolism so that not only did further accumulation of acid bodies cease but the acetone bodies already combined with the base were oxidized thus freeing the base to recombine with CO_2.

This chart and figure 5 in the present review also show other interesting facts concerning insulin. With excess of insulin the [$BHCO_3$] and pH tend to become abnormally high. Such pH studies in stupor from excess of insulin are very few but there are indications in the experiments of Cullen and Jonas that it tends to be associated with an alkalosis (high pH and high CO_2).

One of their patients upon breaking his diet immediately started back toward his initial acidosis but this was easily checked with insulin.

In the data of Cullen and Jonas a tendency to constancy of the pCO_2 rather than of pH is exhibited by a number of individuals, but not in all their cases.

These results are in entire agreement with those reported simultaneously by Bock, Field and Adair (1923) and since by Myers and Booher (1924). It would appear that insulin treatment alone, without alkali is sufficient to restore the acid base balance from diabetic acidosis to a normal state or to one of alkalosis. It is also evident that the human organism can recover after reduction of the serum pH to as low a figure as 7.0.

Rheumatic fever

Because of the old theory that the joint fluid of rheumatic fever was sufficiently acid to liberate free salicylic acid, Boots and Cullen (1922) studied electrometrically and colorimetrically the pH of the joint fluids from such cases. Sterile joint fluids showed a pH approximately

that of normal blood. In a few of these cases the plasma pH was determined and found to be within normal limits (unpublished results). There is therefore no evidence of a disturbance of the acid base equilibrium in rheumatic fever nor of the possibility of the mechanism of salicylate action mentioned above.

Acidosis of children

Howland's and Marriott's (1916) studies indicated that an acidosis is associated with the dehydration of infants. Mitchell and Jonas, (1925) found that although low pH and low [CO_2], i.e., a true acidosis, might be associated with such conditions, such was not invariably present. Further there was no constant relationship between the severity of the disease and the acidosis. They conclude that the acidosis when it occurs is a result of the general derangement and not a causative factor in the condition.

Fasting

Gamble, Ross and Tisdall (1923) have studied the behavior of the acid base equilibrium during fasting in four epileptic children, with reference to the factors regulating excretion of acid and base. Their studies deal also with the changes in alkali reserve of the serum but do not furnish data concerning the pH.

Bigwood (1924b) and Geyelin and Bigwood (in press) have studied the pH and [CO_2] of plasma during the fasting treatment for epilepsy. Koehler (1923) found after fasting 50 hours a mean depression of 5 vols. per cent [CO_2] and of 0.02 in pH. After 77 hours fasting the mean depression in [CO_2] was 8 vols. per cent and in pH a fall of 0.10.

Anoxemia

The influence of anoxemia in inducing acidosis has been a subject of much theorizing. Wallace and Pellini (1921) studied a variety of toxic substances as to their effect upon the alkali reserve. They found that uranium, cantharidin, diphtheria toxin, large doses of arsenic, sodium nitrite when given in a dose large enough to cause methemoglobin formation, and potassium cyanide all produced marked fall in the plasma bicarbonate whereas emetin hydrochloride, hydrazin and

morphin produced no fall of alkali reserve. The first four substances mentioned are capillary poisons. An extremity poisoned with diphtheria toxin when transfused with normal arterial blood returned the venous blood with a lowered alkali reserve and this led the authors to the belief that the acidosis arose from a disturbance of the muscle metabolism. The greater fall in alkali reserve from the first four poisons than following double nephrectomy led them to exclude mere impairment of renal excretion as the important factor in the acidosis. Poisons which like emetin and podophyllin are selective for the intestinal capillaries failed to produce any marked acidosis; nor did marked liver injury from hydrazin cause acidosis. They concluded that after large doses of sodium nitrite with methemoglobin formation, after cyanide which stops tissue oxidation and after the first four poisons mentioned above, which are general capillary poisons, the acidosis results from interference with tissue oxidation and they emphasize the importance of capillary poisons as a cause of acidosis.

This conclusion with regard to cyanide poisoning is confirmed by the work of Holboell (1924), who found that the alkali reserve and pH are reduced in cyanide poisoning and that the venous blood is returned as fully oxygenated as the arterial blood, indicating that the interference with oxygen utilization is in the tissues.

pH, lactic acid and ketonuria

Davies, Haldane and Kennaway (1920) have called attention to the fact that when either from hyper-ventilation of the lungs and consequent decrease in the pCO_2, the pH of the blood is increased, or when from bicarbonate ingestion the alkali reserve is increased with, or possibly without, increase in the pH of the blood, there is a tendency for acetone bodies to appear in the urine. This ketonuria, the mechanism of which is not clear, but occurring during an alkalosis, is evidence of the importance of distinguishing between ketonuria and the state of the acid base balance in the blood. Macleod and his co-workers (1917, 1918, 1921) have shown that administraton of alkali causes an increase of lactic acid in the blood and urine, and an increase of glycolysis in the blood with a decrease in blood sugar concentration. Anrep and Canaan (1923) using a Starling heart-lung preparation and defibrinated blood found that the lactic acid concen-

tration of the blood rose and fell with the pH of the blood and concluded that the pH of the blood regulates the rate of removal of lactic acid. They suggest that this constitutes another factor in addition to the hemoglobin and other proteins and to the bicarbonate and to the phosphates of the blood, in regulating the acid base balance of the blood.

The formation of lactic acid during muscle contraction, a complete review of which is given by Hill (1922), has been demonstrated and studied quantitatively especially by Meyerhof and by Hill. The extent to which lactic acid can influence the alkali reserve and plasma pH during violent exercise has been shown by Barr and his associates (1923) and by Himwich and Barr (1923). The importance of oxygen in the removal of this lactic acid has also been shown by Meyerhof and by Hill but the extent to which disturbance of this mechanism occurs in disease has not been studied. The influence of pH on the recovery process of muscle has been studied by Hartree and Hill (1923, 1924). The direction and possible extent of the disturbance of the acid base equilibrium following exercise is shown in figure 5 from the data of Barr and his associates, and its magnitude is very striking.

Anaphylactic shock

It is interesting to consider the evidence for acidosis in anaphylactic shock. Eggstein (1921a, 1921b), Underhill and Ringer (1921), Hirsch and Williams (1922), Bigwood, Cogniaux and Collard (1924) and Bigwood (1924a) have demonstrated a fall in alkali reserve in the dog and in man during anaphylactic shock and in the condition of low blood pressure following the injection of protein split products, typhoid vaccines and histamine hydrochloride and Hirsch and Williams and Bigwood and his associates find also a marked fall in pH. These authors have observed some parallelism between the degree of shock or fall in blood pressure and the extent of the acidosis. Eggstein found that a fall in the CO_2 capacity of the plasma determined by the technique of Van Slyke and Cullen (1917) to below 25 vols. per cent in his experiments was usually associated with a fatal outcome and that a preliminary administration of sodium bicarbonate before the production of anaphylactic shock diminished the acidosis and lessened the mortality. Bigwood and his associates point out that the fall in

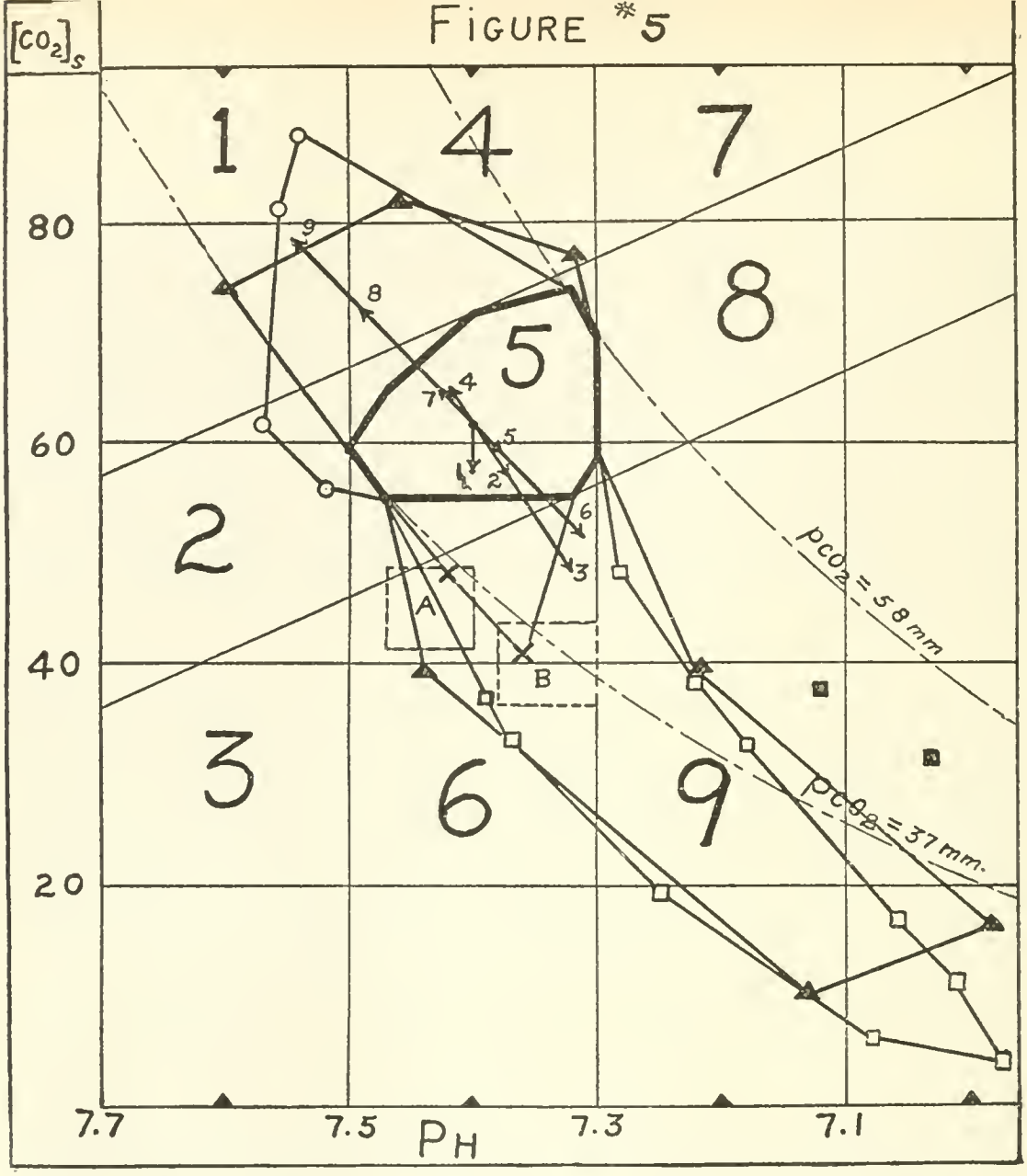
[$BHCO_3$] and in pH involves an increase in the maximal [Ca^{++}] (see our discussion of tetany); a fact possibly of importance in view of Hamburger's evidence for decreased permeability of the capillary wall resulting from increase in [Ca^{++}]. The slope of the plotted data of Bigwood and his associates indicates a change in acid base equilibrium quite similar to that which we have observed from anesthesia. That is, associated with a true acidosis there is somewhat further depression of pH suggesting a greater depression of the respiratory center than occurs simply from administration of acid, or from nephritic or diabetic acidosis.

Traumatic shock

Numerous studies of the alkali reserve in traumatic shock have appeared in the literature which substantially support the findings of Cannon (1918) that there is a lowering of the alkali reserve when there is lowering of blood pressure from shock or from hemorrhage, but his conclusions as to the importance of the acidosis as a cause of the shock have not been established. Raymund (1920) has pointed out that the evdences of shock may precede the fall in alkali reserve. In five dogs traumatized under local anesthesia no striking fall in the alkali reserve appeared until the condition of the animals became quite serious. When general anesthesia is given the effects of the anesthesia upon the acid base equilibrium render the interpretation of the changes due to shock per se very difficult. (See later discussion of anesthesia).

Toxic substances

The administration of methyl alcohol to dogs was shown by Pohl to be followed by greatly increased excretion of formic acid in the urine, the maximum excretion being reached on the third or fourth day. Krol demonstrated a well defined increase in the ammonia and creatin of the urine in dogs in similar experiments. Bongers gave methyl alcohol to dogs and measured the amounts recovered by gastric lavage repeated over several days. He asserts that he recovered about three times as much methyl alcohol in the combined washings in the second and third days as he was able to obtain in those of the first, suggesting very slow metabolism of the alcohol. In spite of this evidence of abnormal acid excretion Loewy and Munzer (1923b)



VAN SLYKE TYPE OF GRAPH SHOWING NORMAL AREA OF FIGURE 4 WITH ITS LIMITING pCO_2 CURVES AND ITS LIMITING CO_2 ABSORPTION CURVES

Large figures are Van Slyke's areas:

1: Uncompensated alkali excess.
2, 3: Uncompensated CO_2 deficit.
4: Compensated alkali or CO_2 excess.
5: Normal balance.
6: Compensated alkali or CO_2 deficit.
7, 8: Uncompensated CO_2 excess.
9: Uncompensated alkali deficit.

Numbered arrows indicate direction and magnitude of change induced by

1. Lactic acid (see table 3); 2. NaH_2PO_4 (table 3); 3. HCl (table 3); 4. Ingestion of 84 mgm. (1 mM) per kilo of $NaHCO_3$; 5 to 6, immediate effect of one hour of radiation (Kroetz, 1924); 7 to 8, day following radiation (Kroetz); 9. One hour of radiation (Hussey, 1922).

○———○ Area including cases of alkali administration or loss of acid by vomiting (Myers and Booher, 1924).

▲——— ▲ Area including diabetics with acidosis (low$[CO_2]$) and under insulin treatment (high $[CO_2]$) (Cullen and Jonas, 1923; Myers and Booher, 1924).

A. Fasting 50 hours (Koehler, 1923).

B. Fasting 77 hours (Koehler, 1923).

□— □ Nephritis (Myers and Booher, 1924; Linder, Hiller and Van Slyke, 1925).

■ Effect of exercise (Barr, Himwich and Green, 1923a).

found in rabbits no diminution in the blood bicarbonate Harrop and Benedict (1920) however in a human case of methyl alcohol poisoning found increase of organic acid in the urine and specifically of lactic and formic acids and in addition a fall of plasma bicarbonate to 36 vols. per cent. Alkali therapy raised the latter to 86 vols. per cent. pH studies are not available. Haskell, Hileman and Gardner (1921) found a reduction in the CO_2 capacity of most of their dogs poisoned with methyl alcohol but not in all, nor was the reduction in CO_2 capacity commensurate with the severity of symptoms. Rabinovitch (1922) found a fall in [CO_2] in one human case to 26 vols. per cent with a rise in blood phosphorus to 11.2 mgm. [P] per 100 cc.

It will be seen from figure 5 that the effects of acid or acid producing salts, of fasting, of diabetic acidosis of nephritic acidosis, of severe exercise and probably of anaphylactic shock is to produce change in the acid base equilibrium in a similar direction and to a degree that is dependent upon the severity of the disturbance.

Tetany

Tetany is a condition intimately related to the acid base equilibrium of the blood. The studies of the pathogenesis of this condition have been recently reviewed by MacCallum (1924) and any extensive repetition of the data there so well presented seems unnecessary. Certain aspects of the condition especially in relation to the pH of the serum may be discussed further here to advantage however.

It is evident from a study of the literature that tetany is associated with one or more of the following:

a. Destruction, removal or disease of the parathyroid glands
b. Increased alkalinity of the serum
c. Diminished [Ca] and increased [P] of the serum

Binger (1917) showed that diminution in the [Ca] and increase of the [P] of the serum produced by injection of phosphate gave rise to marked tetany when the pH of the injected solution was about 6.1 or greater, gave rise to only slight tetany when the pH of the solution injected was 5.8 and to none when its pH was 4.5. With the availability of Cullen's (1922) method for direct measurement of the serum pH the authors in conjunction with H. C. Gram and H. W. Robinson carried out

similar experiments thus far unpublished. Dogs were injected intravenously with (a) M/10 Na_2HPO_4, (b) M/10 NaH_2PO_4, (c) M/10 NaH_2PO_4 + M/10 NaCl. Each dog received 49 cc. per kilo in 53 minutes. Blood was taken from the left ventricle before and two minutes after completing the injection, defibrinated under oil and the serum removed without loss of CO_2. Serum analyses are given in table 4.

The methods used are the same as those employed in studies of ether anesthesia by Austin, Cullen, Gram and Robinson (1924): [Ca] and [P] were analyzed by the methods of Tisdall (1923) and Tisdall (1922b) respectively.

TABLE 4

SOLUTION INJECTED	TIME OF BLEEDING	pH	[$BHCO_3$]	CALCULATED pCO_2	PROTEIN	[Ca] PER 100 CC.	[P] PER 100 CC.	[Cl]
			mM.	*mm.*	*grams per cent*	*mgm.*	*mgm.*	*mM.*
a. Na_2HPO_4 M/10	Before	7.34	19.3	40	7.1	11.1	2.0	111
	After	7.36	20.4	43	5.4	6.6	27.7	95
b. NaH_2PO_4 M/10	Before	7.28	18.9	39	8.1	10.8	4.4	111
	After	7.13	9.6	28	6.7	7.0	66.2	99
c. NaH_2PO_4 M/10, NaCl M/10	Before	7.35	20.1	39	6.7	10.4	3.5	111
	After	7.08	11.2	41	4.3	6.3	49.6	113

In the experiments quoted typical tetany appeared in experiment (a); no tetany was present in (b) and (c). It is evident that the greater tendency to tetany in (a) as compared with (b) is not due merely to the larger amount of Na administered in (a) for in (c) where the Na administered is the same no tetany developed. The difference in tendency to produce tetany in these as in Binger's experiments lies apparently with the resulting pH of the serum or with the pH and bicarbonate concentration of the serum.

Recognition of a relationship of this sort in connection with the physiological activity of Ca together with the recognized limited solubility of Ca led Rona and Takahashi (1913) to attribute the physiological activity of calcium to the calcium ion concentration and,

assuming the blood saturated with $CaCO_3$, to calculate the calcium ion concentration according to the equation:

$$[Ca^{++}] = K \frac{[HCO_3^-]}{[H^+]} \tag{20}$$

Brinkman has included the phosphate ions in a similar equation on the assumption that the blood is also saturated with calcium phosphate. The most carefully developed form of this double equation is that of Kugelmass and Shohl (1924), who have evaluated the constants for these and related equations in aqueous solutions at 38°C. A form of the combined equation given by them is:

$$[Ca^{++}] = \sqrt{\frac{(7.6 \times 10^{-5})\ [H^+]}{[HPO_4^=]\ [HCO_3^-]}} \tag{21}$$

A fundamental limitation of the usefulness of such an equation has not been brought out however in their discussion. Equation (21) is derived by combining the two following equations:

$$[Ca^{++}] = 133 \frac{[H^+]}{[HCO_3^-]} \tag{22}$$

which is the maximum $[Ca^{++}]$ possible in an aqueous solution saturated with $CaCO_3$; and

$$[Ca^{++}] = \frac{(67 \times 10^{-3})}{[HPO_4^=]} \tag{23}$$

which is the maximum $[Ca^{++}]$ possible in an aqueous solution saturated with $CaHPO_4$. In discussing the use of a combined equation such as (21) it has commonly been overlooked that such an equation is valid only when the system is saturated simultaneously with both $CaCO_3$ and $CaHPO_4$ and furthermore that under these conditions the following conditions are obligatory:

1. A fixed ratio must exist for $\frac{[HCO_3^-]}{[HPO_4^=]}$

2. The $[Ca^{++}]$ calculated from equation (22) will equal the $[Ca^{++}]$ calculated from equation (23) and also of course that calculated from equation (21).

Whenever the $[Ca^{++}]$ calculated from equation (22) differs from that calculated from equation (23) then the system is saturated only with

the salt corresponding to the equation giving the lower calculated [Ca^{++}], and that value represents the maximum possible [Ca^{++}] in the system. Under these conditions, equation (21) is no longer valid for it is based on the assumption that the system is saturated with both carbonate and phosphate. The proper use for the equations in so far as the results obtained on pure aqueous solutions of the salts can be applied to serum, would be to use equations (22) and (23) separately and take as the significant figure the lower calculated value as representing the maximum possible calcium ion concentration for the serum. This method we applied to the experimental data given in table 4 with the results shown in table 5. The values in parentheses can have, as pointed out, no real significance. With the assumptions that we are making here, the maximal calcium ion concentration after

TABLE 5

EXPERIMENT		[Ca^{++}] EQUATION (22) (CARBONATE)	[Ca^{++}] EQUATION (23) (PHOSPHATE)	TETANY
a	Before	0.41	(1.24)	
	After	(0.37)	0.09	Marked
b.	Before	0.37	(0.58)	
	After	(1.03)	0.04	None
c.	Before	0.30	(0.71)	
	After	(0.99)	0.06	None

injection is in each instance limited by the concentration of $HPO_4^{=}$ and there is no relation between this maximal [Ca^{++}] and the presence or absence of tetany.

It would seem therefore that the influence of pH upon the development of tetany cannot be accounted for merely by its influence upon the maximal possible calcium ion concentration. It seems probable that the pH has an additional physiological effect in influencing the susceptibility of the organism to the calcium ion concentration or to the balance of the inorganic ions.

Hemorrhage

Wilson (1923) has pointed out that the immediate effect of a large experimental hemorrhage is fall in blood bicarbonate, soon

followed, however, by a rise above normal associated with an increase of pH above normal.

Gastric disorders

In disease of the gastro-intestinal tract the only striking alteration observed in acid-base equilibrium is the alkalosis associated with persistent vomiting and consequent loss of acid from the body in the form of HCl. This alkalosis may give rise to tetany. MacCallum and others (1920) in experimental pyloric obstruction obtained relief of the tetany with injections of NaCl.

MacAdam and Gordon (1922) have reported the finding of an alkalosis in cases of periodic vomiting associated with definite ketonuria. This constitutes another instance of the danger of assuming a condition of acidosis to be present because acetone and diacetic

TABLE 6

	AVERAGE pH
12 normals	7.29
8 miscellaneous diseases	7.33
23 carcinoma cases	7.34

acid are present in the urine. Following prolonged alkaline treatment for gastric or duodenal ulcer, Hardt and Rivers (1923) have reported toxic manifestations associated with rise in the plasma [CO_2] and with evidences of impairment of renal function.

Myers and Booher (1924) report several cases of high alkali both compensated and with high pH following Sippy treatment for ulcer. They also write "we are inclined to think that alkalosis is a condition overlooked and sometimes confused with acidosis by the clinician. We believe great care should be exercised in administration of alkali."

Neoplasms

In cases of carcinoma the serum ash was found by Moore and Wilson (1906) to require slightly more acid for its neutralization than normal serum and this observation was confirmed by Watson (1909). Menten (1917) reported an increase in the pH of serum of carcinoma cases, but later studies by Chambers and Kleinschmidt (1923) in which the pH was calculated from the CO_2 absorption curves and the CO_2 content

of the blood as drawn with correction for oxygen unsaturation shows the averages given in table 6.

Myers and Booher (1924) in a series of 11 cases of uncomplicated carcinoma found no change in the acid base equilibrium, nor did Corran and Lewis (1924).

It seems probable therefore that there is no significant change in the serum pH in carcinoma.

Radiation

The effect of x-ray radiation has been studied in rabbits by Hussey (1922). He observed an increase in both plasma CO_2 capacity and pH by Cullen's method, evident one hour after radiation and still present after 48 hours. The increase in pH in three hours was from 0.11 to 0.18 and in the plasma CO_2 capacity from 16 to 18 vols. per cent.

Kroetz (1924) has found in man after both x-ray and ultraviolet radiation a fall during the first hour of 3 to 10 vols. per cent in the serum bicarbonate and of 0.02 to 0.09 in pH, followed by a rise of both [$BHCO_3$] and pH the following day to about as much above the initial value. These high values may persist for a few days. These changes are consistent in all of his observations, but are obviously small.

Balderrey and Barkus (1924) have observed the effect of exposure to sunlight upon the pH of patients as measured by Cullen's (1922) method. They found no effect on cloudy days but on bright days an increase in the average pH of 0.17. When pigmentation was marked they observed less change in pH.

Reference to figure 5 will show that the blood after administration of alkali and at a certain stage after radiation with ultraviolet light or x-ray is characterized by a change in acid base equilibrium opposite to that produced by administration of acid both as regards [$BHCO_3$] and pH. On the other hand, the rise in bicarbonate seen in certain diabetics treated with insulin shows a tendency to be associated with normal rather than with increased pH.

Surgical anesthesia

In surgical anesthesia we have evidence of a combination of acidosis in the sense we are using the term and of depressed ventilation.

In discussions and studies of acidosis following general anesthesia with or without operation, the evidences of abnormality which have been taken as evidence of acidosis may be considered in three groups.

1. Changes in plasma bicarbonate and pH
2. Ketonuria
3. Certain clinical symptoms

Diminution in the plasma CO_2 during and immediately after ether anesthesia in man and animals has been observed by Austin and Jonas (1917) Caldwell and Cleveland (1917), Carter (1920), Collip (1920), Van Slyke, Cullen and Austin (1922), Leake, Leake, and Koehler (1923), and Austin, Cullen, Gram and Robinson (1924).

The fall of plasma CO_2 in man during operation under general anesthesia has been found to be from about 4 to 10 vols. per cent with return to approximately normal within twenty-four hours. Various types of anesthesia differ only in a minor degree in the amount of fall produced and even in operations under local anesthesia Caldwell and Cleveland observed some fall. Such changes in the alkali reserve are relatively small. In dogs under ether anesthesia the fall may be occasionally considerably greater, amounting in one instance to 22 vols. per cent (Van Slyke, Cullen and Austin (1922)), but as a rule in dogs also the fall is less than 10 vols. per cent.

The change in pH has been less extensively studied. Menten and Crile (1915) reported a fall in the blood pH in rabbits and Van Slyke, Cullen and Austin (1922) demonstrated fall in pH in the dog under both light and deep ether anesthesia. Leake, Leake and Koehler (1923) confirm the finding. Cullen, Austin, Kornblum and Robinson (1923) have shown that this fall occurs chiefly during the early minutes of anesthesia and Austin, Cullen, Gram and Robinson (1924) have demonstrated that it is due chiefly to the introduction of some unidentified acid into the blood, together with Cl^- anions. The possibility that the unidentified acid is lactic acid has not been excluded. That lactic acid formation in the muscles is actually largely responsible for the fall in alkali reserve in the dog under ether anesthesia has now been demonstrated by Ronzoni, Koechig and Eaton and they have also discussed a possible relation of this with the observations of Stehle and Bourne (1924).

Stehle and Bourne (1924) have shown following ether anesthesia an increased phosphorus elimination in the urine, a 2.5 per cent decrease in muscle phosphorus and a 12.5 per cent increase in liver phosphorus. The change in blood phosphorus, however, is slight which taken into consideration with the base findings of Austin, Cullen, Gram and Robinson (1924) indicates that the explanation of the diminished alkali reserve is not a withdrawal of base from the blood consequent upon mobilization of muscle phosphoric acid. On the other hand the view advanced by Y. Henderson and Haggard (1918) that the fall in alkali reserve was a compensatory phenomenon consequent upon hyperpnea and excessive removal of CO_2 in the early stages of anesthesia has not been supported by the other studies mentioned.

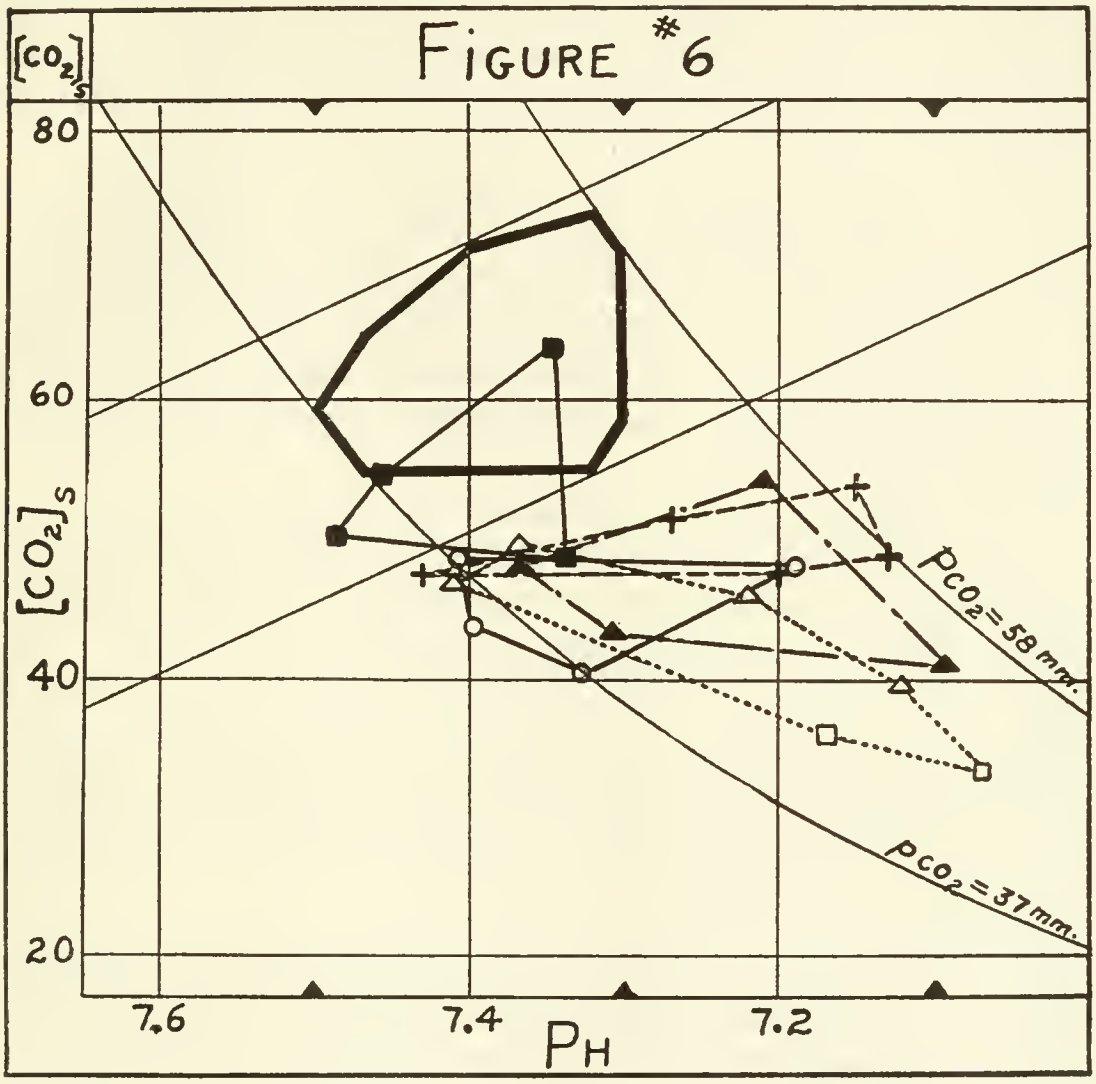
Koehler (1924) has studied the acidosis of anesthesia as it occurs in human subjects. The changes observed are comparable to those found in experimental studies. Recovery of the acid base balance is fairly rapid and usually complete in from one to three hours. Koehler divides the recovery into two phases: that from excessive pCO_2 which is rapid occurring within the first hours and that from depressed alkali reserve which is slower.

Leake and Hertzman (1924) have studied the effects of ethylene-oxygen and nitrous oxide-oxygen anesthesia on the acid base equilibrium. They observed changes in the acid base equilibrium similar to but less marked than those observed with ether and chloroform. In addition, the factor of anoxemia is of great importance in these types of anesthesia. There may be an initial increase in pH if the anoxemia is marked. Leake (1924) has also discussed the disturbances of carbohydrate metabolism following ether anesthesia.

The studies of White (1923) upon the beneficial effect of post-operative administration of CO_2 as a respiratory stimulant to accelerate the removal of ether through the lungs, a procedure which in itself necessarily tends for the time toward still further lowering the pH, indicate that the fall in pH is of less importance than the other effects of the ether. The procedure adopted by White led to a diminution of disagreeable post-operative symptoms.

That ketonuria as shown by qualitative tests for acetone and diacetic acid is a common finding after surgical anesthesia has long been noted and has been taken as evidence of acidosis. Reimann

and Bloom (1918) and Caldwell and Cleveland (1917) demonstrated that ketonuria could often be detected immediately before operation,



VAN SLYKE TYPE OF GRAPH SHOWING NORMAL AREA OF FIGURE 4 AND EFFECTS OF ANESTHESIA

(From data of Cullen, Austin, Kornblum and Robinson (1923) in dogs and of Koehler (1924) in human cases.)

■——■ Before anesthesia.
+——+ Human cases under nitrous oxide.
▲——▲ Human cases under ether.
○——○ Human cases after nitrous oxide.
Δ.....Δ Human cases after ether anesthesia.
□——□ Dogs after ether anesthesia.

probably due to the preliminary fasting, but that it was much more frequent following the operation. On the other hand, they established the fact that there was no constant relation between the severity or

duration of the operation or the seriousness of the post-operative course, or the degree of reducton in the alkali reserve on the one hand, and the degree of the ketonuria on the other. Indeed, it seems possible that the ketonuria is mainly an expression of the fasting incident to operation, and there is no evidence that it is of any serious clinical importance.

Of interest in this connection, however, are the observations of Thalhimer (1923), of Fisher and Snell (1924) and of Ginsberg (1924) upon the effect of insulin and carbohydrate given after operation, with a reported disappearance of ketonuria and lessening of disagreeable post-operative symptoms. The experimental studies of Stewart and Rogoff (1917) and of Ross and Davis (1920) are of especial interest in this connection.

A third group of phenomena described following operation and sometimes attributed to acidosis, probably chiefly because the common ketonuria has been examined for and noted in these cases, is a clinical picture which is essentially that of post-operative shock. The evidence that these cases actually suffer from acidosis is however lacking. These cases are not numerous in the surgical clinics and the opportunities for accurate studies of the blood are still rarer. In two such cases studied by the authors and believed by the surgeons to be post-operative acidosis, both showed plasma [CO_2] value above normal.

It seems probable therefore that grouped under the name of acidosis three more or less distinct pathological conditions have been recognized as occurring at times after general anesthesia with or without operation. Of these, the one of chief clinical importance is that characterized above as a form of post-operative shock, but this condition is probably often not accompanied by acidosis and is probably not due to acidosis.

The ketonuria so often observed after operation probably arises in many instances from the fasting preceding and following operation. In itself it is of doubtful clinical importance. It may be associated with alkalosis rather than with acidosis. The value of treatment with carbohydrate with or without insulin must be based upon the effect of such treatment on the post-operative symptoms.

The fall of plasma bicarbonate and of pH, which alone should be

called acidosis, appears to be a constant accompaniment of experimental and surgical ether, chloroform and nitrous oxide anesthesia, but there is no satisfactory evidence that it is of clinical importance in human surgery. When the disturbances of the acid base equilibrium that occur normally with vigorous exercise, as shown by the studies of Barr and his associates (1923) are called to mind, it may well be questioned whether the exertion incident to passing under the anesthetic may not be an important factor in this acidosis exaggerated possibly by interference under the anesthetic with normal tissue oxidation. It is not at all clear that the acidosis is a menace to the individual or that alkali therapy is indicated. With removal of the anesthetic spontaneous restoration of the acid base equilibrium is rapid as is the case after vigorous exercise. Failure of the acid-base equilibrium to recover after operation occurs probably only incident to some serious disturbance of the metabolism, and is not so far as we know a consequence of the acidosis per se.

Lobar pneumonia

In certain infectious diseases various workers have reported disturbances of the acid base equilibrium. The most thoroughly studied infection is lobar pneumonia.

Palmer (1917) demonstrated the excretion in the urine in lobar pneumonia of large amounts of organic acid which at a pH of 5.0 was present as free acid. At this pH, 87 per cent of uric acid is free, one-third of β-hydroxybutyric and of acetic but only about 5 to 7 per cent of hippuric, diacetic and lactic acids. This fact excludes the last three from consideration as the acid excreted in pneumonia. Palmer excluded by analysis β-hydroxybutyric acid, uric acid and ethereal sulphates. The nature of the acid was not further identified. There may be a relation between these observations and those of Barach, Means and Woodwell (1922) who observed a tendency to lowering of the alkali reserve in pneumonia. They found in 10 cases an average CO_2 capacity in arterial blood at 40 mm. pCO_2 at 37° of 43.2 vols. per cent as compared with the average for normal individuals found by Peters, Barr and Rule (1921) of 49.3 vols. per cent; the lowest observed in pneumonia was 35.0 vols. per cent. Barach, Means and Woodwell calculated the arterial pH from the CO_2 absorption curve and obtained

an average in their 10 cases of pneumonia of 7.31, with four cases below 7.30 which they take as the lower limit of normal resting pH. The lowest pH they observed was 7.20. They found no relation, however, between the pH or the reduction in the alkali reserve and the prognosis or the degree of anoxemia. They observed a spontaneous rise in the alkali reserve and return of pH to normal at, or shortly after, crisis in three patients studied before and after crisis. In another patient they observed the same restoration without crisis after vigorous oxygen therapy. Binger, Hastings and Neill (1923) have reported edema occurring during convalescence from pneumonia in association with bicarbonate administration. Hastings, Neill, Morgan and Binger (1924) have more recently studied a series of 30 pneumonia patients, making direct determination of the pH and [CO_2]. They observed a lower arterial CO_2 tension during the febrile period than after return to normal temperature in seven cases but lowered pCO_2 and increased oxygen unsaturation did not occur together with sufficient regularity to indicate a causal relationship. They noted no tendency towards an acidosis of either metabolic or respiratory origin. The alkali reserve was within or near normal limits in every case and the pH was in each instance within normal limits, 7.30 to 7.50, and in most instances in the more alkaline half of this range. Their results contraindicated alkali therapy in all the cases studied. In 8 of the 10 cases in which arterial oxygen saturation was determined an abnormally low saturation was observed at some stage of the disease. Taken with the non-occurrence of increased CO_2 tension these results they concluded support the view that when the mechanism for gas exchange in the lungs is affected, absorption of oxygen fails before elimination of carbon dioxide is significantly impaired.

Disturbances similar to those produced by hypopnea or hyperpnea

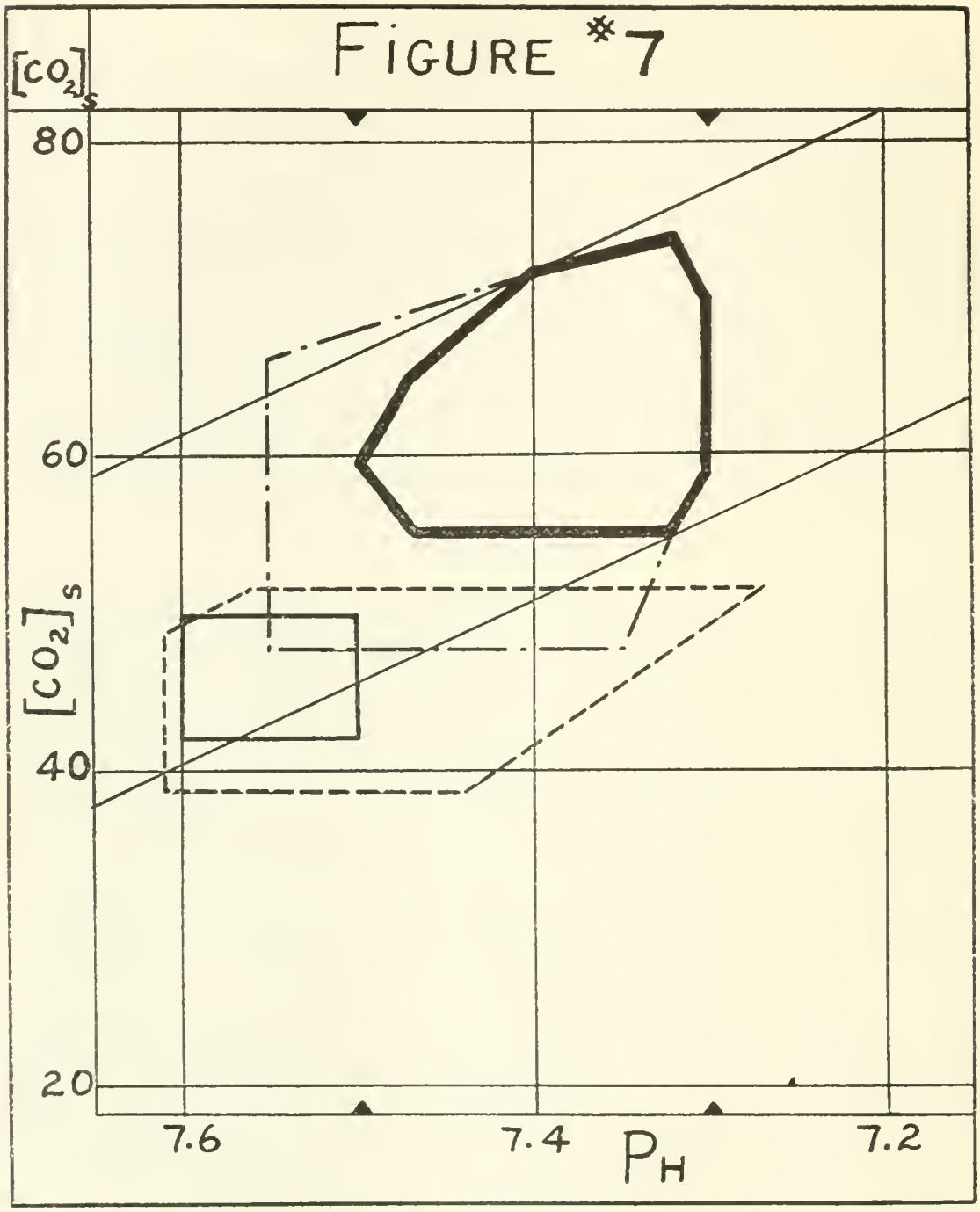
Disturbances in the excretion of CO_2 either by a hyperpnea leading to a lowering of the alveolar pCO_2 or by impaired pulmonary ventilation with rise of pCO_2 produce changes in the blood that have been extensively studied. The subject has been thoroughly and extensively reviewed by Van Slyke (1921a, 1921b) with regard to the mechanism by which the pH of the blood is related to its pCO_2, and a discussion here of the development and present knowledge of this phase of the

subject would therefore be superfluous. The relation of changes in pH of the blood to heart, bloodvessels and the centers of the central nervous system has been touched upon already.

In the study of disease so long as we were limited to the measurement of the plasma or blood [CO_2] and to the direct measurement of the alveolar pCO_2 with its well recognized difficulties in untrained or very ill patients the recognition of disturbances in acid base equilibrium consisting chiefly of hypoventilation or of hyperventilation was difficult. With the increasing data on both plasma or blood [CO_2] and direct measurement of pH of the serum there appears to be emerging evidence of disturbances of this type in certain diseases.

Infections

Hachen and Isaacs (1920) and Koehler (1923) have found in influenza, influenzal bronchopneumonia and grippe with temperatures above 103° and the latter in one case of peritonitis a reduction in the CO_2 capacity of the plasma and CO_2 content of the blood which may be rapid or gradual in its development. Koehler found the pH of the venous blood, however, increased rather than diminished and believes the fall in blood bicarbonate may be at least in part compensatory to a febrile hyperpnea, analogous to the shift in acid base equilibrium which Bazett and Haldane (1921) observed on immersion in a hot bath and which Koehler (1923) confirmed. The latter's observations are plotted in figure 7. Yamakita (1921) however, observed a fall in alkali reserve in experimental infections even when the temperature was not very high but only with very great increase in temperature in hyperthermia due to heat puncture. Hirsch and Williams (1922) found in rabbits infected by intravenous injection of pathogenic bacteria a marked lowering of both CO_2 capacity of the plasma and of pH and Leake, Vickers and Brown (1924) observed similar changes in experimental B. bronchisepticus pneumonia in dogs. The association of the fall in [$BHCO_3$] in experimental pneumonia and other experimental infections with a lowered pH suggests that febrile hyperpnea is not always the important factor in the shift of acid base condition but that there may be depletion of the alkali reserve in certain infections. The possibility of a renal factor is to be considered in these infections.



VAN SLYKE TYPE OF GRAPH

Normal area of figure 4.

Area of febrile cases (Koehler, 1923).

Area of individuals in hot baths (Koehler, 1923).

Area of individuals in last sixteen weeks of pregnancy (Marrack and Boone, 1923).

The abnormal areas lie almost wholly within the normal CO_2 absorption curves but at lower CO_2 tension. (Van Slyke's "uncompensated CO_2 deficit.")

Reports of ketonuria in the course of infections cannot be taken as evidence of acidosis. Ketonuria indicates a disturbance of the normal fat metabolism. In diabetes mellitus this is constantly associated with acidosis. Ketonuria, however, can be produced by administration of alkali as already pointed out and in the discussion of the effects of ether anesthesia we have pointed out that ketonuria is not proportional to the acidosis present.

Pregnancy

The association of toxemias of pregnancy with increased NH_3/N in the urine has long been noted. Losee and Van Slyke (1917) showed however that in pregnant patients with pernicious vomiting and strikingly high ammonia figures the plasma bicarbonate may indicate no greater degree of acidosis than may be observed in non-toxic pregnancy. Sellards (1912) reported a similar case with only a normal tolerance for sodium bicarbonate. Losee and Van Slyke, (1917) Williamson (1923), Cook and Osman (1923), and Marrack and Boone (1923) have shown some diminution of the bicarbonate of the blood in normal pregnancy. The latter authors in seventeen cases in the last sixteen weeks of pregnancy found bicarbonate of 47 to 65 vols. per cent as compared with 58 to 65 vols. per cent for normals. Cook and Osman found the serum bicarbonate in pregnant women to be on the average 85 per cent of that for normal non-pregnant women. Hasselbalch and Gammeltoft (1915) noted a greater lowering in alveolar pCO_2 in the later months of pregnancy than in the bicarbonate, and Marrack and Boone observed the same. Consistent with this is the observation by Marrack and Boone of a pH of 7.35 to 7.55 by Cullen's method (1922) in their pregnant cases, as compared with 7.30 to 7.45 for normal cases (see fig. 7). This would indicate a tendency to increased pulmonary ventilation in late pregnancy, and the slight lowering of the $[BHCO_3]$ may well be considered, therefore a secondary effect of hyperventilation rather than as due to true acidosis.

We are indebted to Mr. H. W. Robinson for assistance in the preparation of the graphs.

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ANOXEMIA IN LOBAR PNEUMONIA

Its Causes, Pathogenesis, and Significance

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I. THE OCCURRENCE OF ANOXEMIA IN LOBAR PNEUMONIA. THE GENERAL PHYSIOLOGICAL CAUSES AND RESULTS OF ANOXEMIA

Acute lobar pneumonia became more than one hundred years ago recognized as a nosologic morbid entity through investigators belonging to the so-called anatomic school. Our conception of this disease passed unchanged even the bacteriologic era during which a variety of morbid entities mainly built on their symptomatology became split and collected into new entities. In spite of the fact that there are but few diseases which in respect to symptomatology, diagnosis, and treatment are more familiar to the clinician than lobar pneumonia, it is true that no other respiratory disease has during the last few years given rise to more varying, more scientifically fundamental, and more practically important problems.

The recent progress in the study of the etiology and epidemiology of lobar pneumonia and the specific and prophylactic treatment based upon these studies will not be entered upon in spite of the fact that the most important problems concerning lobar pneumonia are of etiologic and epidemiologic nature. We shall confine ourselves to a discussion of the pathological physiology of the disease, where we face questions which scientifically and practically are of almost as much

[1] The public competition for the chair of Internal Medicine at the University of Copenhagen held in September, 1923, required of each candidate four lectures, two to be given on two successive days on a subject chosen by the candidate, one a clinical lecture on a patient assigned by the committee the day before, and one on a subject also assigned the day before and common for all the candidates. The two lectures given here were chosen by the candidate. In the translation from Danish the original form is preserved as completely as possible.

importance as the etiological problems. As long as lobar pneumonia exists—and there is no reason to believe that this disease belongs to the exterminable group of infectious diseases—it is important to study the degree of the functional disturbances caused by the infection. Because, if a functional disturbance affects an organ, the functional power of which cannot be reduced beyond certain limits without serious danger for the body, this disturbance may be of vital importance for the course of the disease. Treatment of such functional disturbance may, therefore,—although of symptomatic nature—prove to be not less important than the specific and prophylactic treatment.

By far the most important functional disturbances, which occur in patients suffering from lobar pneumonia, concern respiration and circulation. In this paper certain disturbances in the function of the lungs will be discussed with special reference to the nature and cause of these disturbances and to their significance for the body as a whole. The main respiratory functions of the lungs—intake of oxygen and output of carbon dioxide—are often disturbed in patients with lobar pneumonia. We shall in this lecture confine ourselves to a discussion of disturbances in the oxygen intake and discuss questions concerning the carbon dioxide only in case they are of importance for the oxygen intake.

Oxygen content of arterial blood in patients with lobar pneumonia

Investigations published within the last few years have shown that the arterial blood, in a large percentage of cases, contains less oxygen in proportion to the hemoglobin than does arterial blood from normal individuals. Or, in other words, the arterial saturation is abnormally small in a number of patients with lobar pneumonia.

Table 1 gives the percentage of oxygen saturation of the arterial blood in 50 patients, compiled from eight different publications. In 12 instances the degree of saturation was found to be between 90 and 100 per cent, which is either normal, or at most, only slightly diminished. In 22 instances the degree of saturation fell between 80 and 90 per cent, in 9 instances between 70 and 80 per cent, and in 7 instances between 60 and 70 per cent.

TABLE 1

Oxygen saturation of the arterial blood in 50 patients with lobar pneumonia and in 14 patients with influenzal-pneumonia

AUTHOR	YEAR	CLINIC	NUMBER OF PATIENTS EXAMINED	OXYGEN SATURATION OF ARTERIAL BLOOD					REMARKS
				100–90 per cent	90–80 per cent	80–70 per cent	70–60 per cent	< 60 per cent	
Hürter	1912	Marburg University	2		1(1)*	1			
Stadie, I	1919	Rockefeller Hospital	6	2	2	2(1)			
Meakins	1921	Edinburgh University	10	2	8				Oxygen treatment
Barach and Woodwell	1921	Harvard University	6	2	2	2			Oxygen treatment
Stadie, II	1922	Rockefeller Hospital	5			2(1)	3		Oxygen treatment
Le Blanc	1922	Eppendorf Hospital	6	1	4(1)	1(1)			
Binger a. o.	1923	Rockefeller Hospital	1				1		Oxygen treatment
Hastings a. o.	1923	Rockefeller Hospital	14	5(1)	5(3)	1(1)	3		Oxygen treatment
Total			50	12	22	9	7		
Mortality			11	1	5	5	0		
Stadie, I. Influenzal-pneumonia	1919	Rockefeller Hospital	26	6	7	7	2	4	
		Mortality	14	0	2	7	1	4	

* Figures in parentheses indicate dead.

The various columns in figure 1 correspond to the average values for the different groups given in table 1. The light part of the column indicates the oxyhemoglobin, whereas the dark part represents the reduced hemoglobin.

The degree of arterial oxygen saturation has been shown to vary considerably during the course of the disease. So far the determinations are too few to justify an attempt to draw parallels between the degree of oxygen saturation and the various clinical and anatomical stages of the disease.

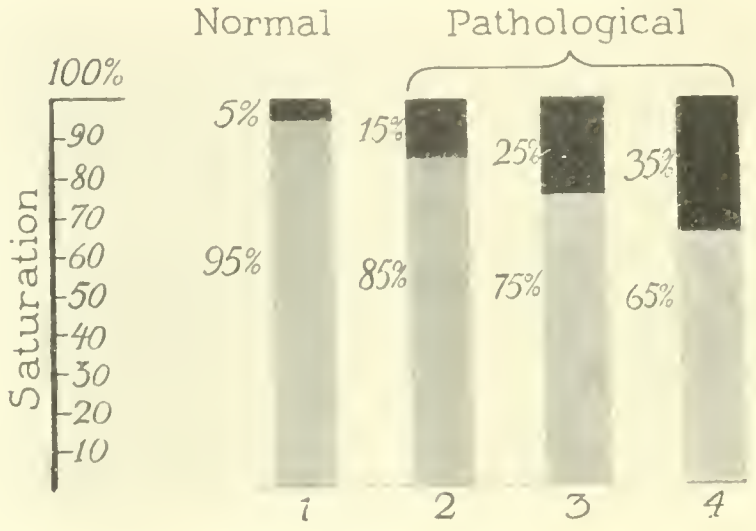

Fig. 1. Diagram of the Arterial Oxygen Saturation of the Various Groups of Patients in Table 1

Dark areas indicate reduced hemoglobin. Light areas indicate oxy-hemoglobin

In table 1 below is given the arterial oxygen saturation in a series of patients with influenzal-pneumonia, where- as we shall briefly discuss later— a decreased arterial oxygen saturation is still more commonly seen than in patients with lobar pneumonia.

The cause of the diminished oxygen content of the arterial blood in lobar pneumonia

In order to facilitate a discussion of the cause of the diminished arterial oxygen saturation in pneumonia, it might be useful first to call attention to the three diagrams in figure 2, where the ratios of

reduced hemoglobin to oxyhemoglobin in the various parts of the circulatory bed are given.

Diagram *A* represents the conditions found in normal individuals at rest. The blood enters the arteries normally saturated with oxygen (about 95 per cent). In the tissue capillaries about one-fourth of the oxygen goes out to the tissues so that the venous blood becomes only three-fourths saturated, which with an oxygen capacity of 20 volumes per cent corresponds to an oxygen content of 15 volumes per cent. Diagrams *B* and *C* indicate the two ways in which the arterial

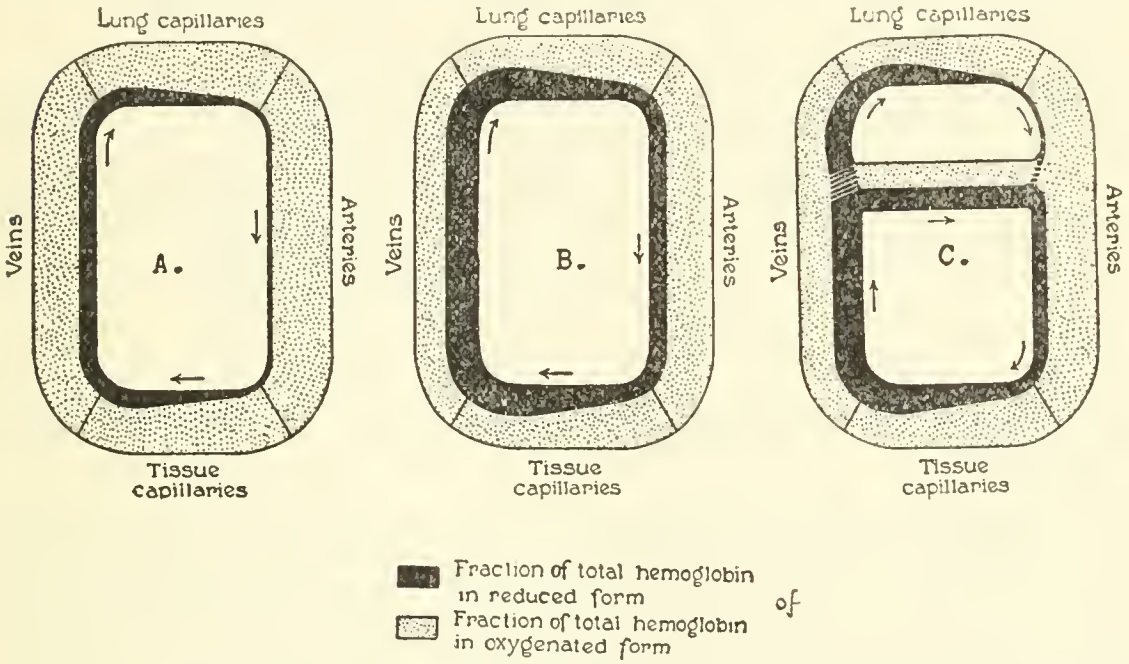

FIG. 2. DIAGRAMS SHOWING THE PROPORTION OF OXYGENATED HEMOGLOBIN TO REDUCED HEMOGLOBIN IN DIFFERENT PARTS OF THE CIRCULATORY SYSTEM

A, in normal individuals; *B*, in a case of incomplete oxygenation in aerated parts of the lungs; *C*, in a case where a fraction of blood passes through completely unaerated parts of the lungs.

oxygen content may be diminished by pathological lung conditions. In *B* all the blood is oxygenated, but to a lower degree than usual, because less oxygen for one or another reason passes from the alveoli to the blood streaming through the lung capillaries. In *C* the arterial oxygen saturation decreases because a fraction of the blood passes through a part of the lung to which air has no access.

It might seem most natural to presume that the cause of the low arterial oxygen saturation so often found in lobar pneumonia is that a

fraction of the blood passes the consolidated area to which air has no access (diagram *C*). Various facts can, however, hardly be brought in accordance with such a conception. First, the dry bloodless condition of the consolidated parts of the lungs, particularly in the stage of gray hepatization, points against any considerable perfusion of the consolidated area. Secondly, as Kline and Winternitz, and later Gross showed, only the largest branches of the *arteria pulmonalis* could be injected with an emulsion of either Berlin blue or barium gelatine. Third, extensive consolidation of the lung tissue is found without any decrease in the oxygen content of the arterial blood.

TABLE 2

Comparison between number of lobes consolidated and degree of arterial oxygen saturation in 14 patients with lobar pneumonia (Rockefeller Hospital)

NUMBER OF LOBES CONSOLIDATED	ARTERIAL OXYGEN SATURATION, PER CENT
1	82.1–84.0–90.2–91.6–97.2*
2	68.0–75.8–86.5–94.4–97.0
3	64.6–68.2*–90.5
4	92.7

* Indicates that the patient was treated in oxygen chamber when the arterial blood was drawn.

In table 2 is given the degree of arterial oxygen saturation in per cent of capacity and the number of affected lobes in a series of patients from the Rockefeller Hospital examined by Hastings, Neill, Morgan and Binger. Besides the routine stethoscopical examinations, roentgenograms were made in all instances. No parallelism between degrees of arterial oxygen saturation and extent of consolidation is seen in these patients. Fourth, in a number of cases it has been possible by oxygen therapy completely to oxygenate the abnormally unsaturated arterial blood. In such cases it can be excluded that any blood has passed a part of the lung to which air has no access (anatomical shunt). The decreased arterial oxygen saturation found in pneumonia must therefore at least in a certain number of cases be explained in another way.

English investigators especially Haldane, Meakins and Priestly, consider shallow breathing mainly responsible for the decreased arterial oxygen saturation When the depth of respiration becomes so small

that only the dead space, which is about 150 cc., is ventilated, the air in some or all alveoli must be renewed by diffusion alone. The normal alveolar oxygen tension is not maintained, and if the oxygen tension decreases sufficiently, incomplete saturation of the blood passing through the lung capillaries may result. Figure 2, *B*, indicates such a condition. This theory cannot be said to be absolutely new in principle, because a similar explanation has been given for the dyspnoea of certain heart patients. However, the English investigators have not only gone deeper into the problems than before, they have also produced experimental support for a theory which previously had been a mere hypothesis. In the first place it has been shown by these three investigators that superficial lung ventilation, artificially produced in normal subjects by means of the so called concertina, would cause periodic respiration, which is known to be due to oxygen want. Secondly, Meakins and Davies have shown that shallow respiration may cause decreased arterial oxygen saturation in normals. They found, for instance, that the arterial blood was 94.3 per cent saturated during normal respiration. If the tidal air was diminished from the normal value of 500 cc. to 315 cc. the arterial oxygen saturation decreased to 91.7 per cent. Further decrease in the tidal air caused periodic respiration. Thirdly, Meakins has determined the depth of respiration and at the same time observed the skin color in 4 patients with lobar pneumonia.

He found that the color became cyanotic if the depth decreased to about 250 cc. (table 3). Binger, Hastings, and Neill observed the depth of respiration, the arterial unsaturation and the skin color in a patient with lobar pneumonia. They found, as shown in table 4 that the arterial oxygen saturation rose and the cyanosis disappeared on increasing the depth of respiration. On the other hand, it does not seem likely that shallow breathing is the only cause responsible for the decreased arterial oxygen saturation in patients with pneumonia. It is beyond doubt that cyanosis of respiratory origin may occur in patients where the depth of respiration is not below normal. Stadie, in a discussion of this problem, puts very little stress on shallow breathing as the cause of low arterial oxygen in pneumonia. It can furthermore not be excluded *a priori* that rapid and shallow breathing may be the result and not the cause of low arterial oxygen in pneumonia.

At any rate, another explanation than shallow breathing, must be found for the low arterial oxygen in a certain number of patients with pneumonia. It is not unlikely that the cause is decreased diffusion from the alveolar air of normal composition to the blood in the lung capillaries. Such a diminished diffusion may be brought about in two ways: first, by the presence of a layer of fluid on the inside of the alveoli, as has been suggested by Hoover as the cause of the cyanosis

TABLE 3

Relation between depth of respiration and skin color in 4 patients with lobar pneumonia (Meakins)

NUMBER OF PATIENT	DEPTH OF RESPIRATION	CYANOSIS
	cc.	
1	270	0
	230	+
2	290	+
3	270	0
	240	+
4	260	0
	230	+

TABLE 4

Relation of arterial oxygen saturation and skin color to depth of respiration in a patient with lobar pneumonia (Binger, Hastings and Neill)

DEPTH OF RESPIRATION	ARTERIAL OXYGEN RESPIRATION	CYANOSIS
cc.	*per cent*	
176	61.0	+
450	91.7	0
540	96.0	0

in bronchopneumonia, and by Barcroft as the cause of the cyanosis in cases of gas poisoning during the war. Second, such a diminished diffusion may be due to a functional disturbance of the alveolar epithelium as proposed by Brauer as an explanation for the cyanotic skin color in certain cases of bronchopneumonia without organic pathological findings. This (hypothetical) condition Brauer has termed pneumonosis, analogous to the term nephrosis, which Friedrich

Müller introduced to indicate certain kidney conditions. Schjerning has produced lung edema in cats and dogs by intravenous injection of chloramine and subcutaneous injection of urethane and also by chlorine inhalation. Blood gas analyses showed incomplete oxygenation of the arterial blood. In two dogs the anatomical changes in the lungs after chlorine inhalation were so slight that he assumes a functional disturbance of the epithelium (a pneumonosis) as the cause of the incomplete arterial oxygen saturation. In human lungs the condition of a diminished diffusion has so far not been experimentally proven. Diagram B in figure 2 indicates in a schematic way the relationship between reduced and oxygenated hemoglobin in the various parts of the circulatory system if the diffusion through the alveolar walls is diminished.

The effects on the organism of incomplete oxygenation of the arterial blood

After having described the existence of incomplete oxygen saturation of the arterial blood and considered its causes, it remains to discuss its effect on the organism. Unfortunately studies correlating blood gas analyses with clinical symptoms are still so few that it seems for the time being impossible to obtain sufficient insight by these means alone. For many years however, physiologists have studied experimentally similar but much less complicated conditions. These investigations may prove of benefit to us and serve as a starting point for a discussion of the complicated conditions which we face when trying to unravel the influence on the organism of insufficient oxygenation of the arterial blood.

The effect of grave disturbances in the oxygen supply

Complete prevention of oxygen supply causes in all higher animals death in a few minutes because of the small amount of reserve oxygen stored in the body. Grave but not immediately deadly disturbances in the oxygen supply cause, as shown in animal experiments, marked irritation of the medulla spinalis and medulla oblongata. Mathison showed for instance in 1909 in decapitated cats that insufficient oxygen supply causes irritation of the skeletal muscles and the vasomotor muscles. Mathison thinks that oxygen lack acts as a stimulus to the

nerve centers, similar to the action of carbon dioxide and lactic acid. He challenges the conclusions of Béthe and of Lindhard that oxygen lack exercises its effect by increasing the irritability of the nerve centers. Another remarkable effect of oxygen lack is bradycardia. This was first shown by Sherrington. Later Th. Lewis and Mathison showed that the slow heart action was caused by a temporary heart block.

Effects of oxygen lack of moderate severity

These effects have been studied in mountain expeditions, in experiments with low atmospheric pressure in air cabinets, in the respiration of air with low oxygen content, in carbon monoxide poisoning, in certain heart diseases, and in severe anemias. The symptoms which can be ascribed to lack of oxygen are in principle the same in all these various physiological and pathological conditions, although they may be modified more or less by circumstances of secondary nature, such as the duration of oxygen lack, which we shall discuss later.

The results in mountain expeditions and in air cabinet experiments are for historic and physiological reasons particularly fit as examples of the effects of oxygen lack of moderate severity. Furthermore the oxygen lack is in these conditions of the same type as in pneumonia in that it is of respiratory origin. We shall therefore in the following mainly confine ourselves to these conditions. The condition which may be the result of oxygen lack is now generally termed anoxemia. This name is a modification of the term anoxyhemia barometrica introduced in 1864 by Jourdanet. By this term Jourdanet expressed that asphyxia of the body tissues which he considered to be present during stay in rarified air or in air with low percentage of oxygen. Anoxemia reveals itself through various symptoms discussed below not all of which are of the same importance or of the same constancy.

Cyanosis

The most conspicuous symptom is cyanosis. For a purely clinical estimation of the presence of anoxemia or of the grade of the condition if present, the cyanotic color is of no less importance than the pallor in anemia or the yellowness in icterus. The essential cause of the cyanosis is the dark color of the non-oxidized hemoglobin present in

the blood. When the color of the blood in the capillaries becomes sufficiently dark it is recognized as a diffuse color change through the skin as through milk glass. Although it is true that the essential cause of cyanosis is the dark color of the reduced hemoglobin, it must not be overlooked that its appearance and degree, if it is present, are influenced by a series of so called modifying factors. Of these factors the state of the capillaries is particularly important. For that reason one can draw only roughly quantitative conclusions from the intensity of cyanosis as to the degree of oxygen saturation. The minimum

TABLE 5

Arterial oxygen saturation at sea level and at the Andes mountains. Oxygen tension in alveolar air and in arterial blood at the Andes. (After Barcroft, 1922)

INDIVIDUAL	ARTERIAL OXYGEN SATURATION		ALVEOLAR OXYGEN TENSION— THE ANDES MOUNTAINS	OXYGEN TENSION IN ARTERIAL BLOOD— THE ANDES MOUNTAINS
	Sea level	The Andes mountains 4300 meter		
	per cent	*per cent*		
M.	95	83–91	56	58
R.	97	87.5	59	52
Bi.		84		
Bo.	95	82		
M. C. Q.		86	59	57
P.		91	55	48
C.		87	54	55
M. C. L.		86	56	47
Z.		86	51	50
V.		82.5		50
B.		83.5		40

concentration of reduced hemoglobin in the capillary blood which produces visible cyanosis can therefore be stated only approximately. However, ordinarily it is not far from 6 or 7 volumes per cent.[2] This corresponds in patients with normal hemoglobin to an arterial oxygen saturation of 80 to 85 per cent. One would therefore expect that normal individuals exposed to rarified air at high altitudes would become

[2] It is convenient to express reduced hemoglobin concentration in terms of oxygen unsaturation, since oxygen values are those experimentally determined. One cubic centimeter of oxygen combines with 0.75 gram of hemoglobin. So that 5 grams of reduced hemoglobin may be expressed as 5/0.75 = 6.7 volumes per cent of oxygen unsaturation "Volumes per cent" expresses, as usual, cubic centimeters of a gas in 100 cc. of a fluid.

cyanotic when the arterial oxygen saturation decreases to about 80 to 85 per cent. As a matter of fact this was found to be the case by Barcroft and his collaborators in the expedition to the Andes in 1922. They found that a cyanotic color began at about 4000 meters. Determinations made at the same altitude showed an arterial oxygen saturation of from 82 to 91 per cent and in most cases around 85 per cent. The figures are given in table 5. In 1915 Hasselbalch and Lindhard in an air cabinet experiment at the Finsen Institute in Copenhagen in a normal individual found no cyanosis at a barometric pressure of 552 mm. corresponding to an altitude of a little less than 3000 meters. If, however, the pressure was lowered to 484 mm. equivalent to an altitude of about 4000 meters cyanosis was found as an inconstant symptom.

Cerebral and cerebellar symptoms

Under anoxemic conditions a variety of psychic disturbances are described. As in the case of the cyanotic skin color, these symptoms do not appear before a certain grade of oxygen lack is present. As a whole it may be ascertained from the reports of various mountain expeditions and air cabinet experiments that such symptoms start at an altitude of approximately 4000 meters, or at a pressure of not far from 500 mm., which corresponds to an arterial oxygen saturation in normals of approximately 85 per cent. Most of the psychic disturbances occurring in anoxemia are of an indefined diffuse nature and can hardly be localized to any certain areas in the brain. Loss of self control as in alcohol intoxication, has been repeatedly observed, for instance by Barcroft at an altitude of about 4000 meters in the Alps. It has also been described by Zuntz and collaborators. By Mosso, and by the expedition to Pike's Peak in 1913, it was noticed that the men often became unreasonable at about the same altitude. Haldane, Kellas and Kennaway reported loss of memory and a delirious condition in an air cabinet at a pressure corresponding to 7500 meters altitude. Sleeplessness and headache were observed by Lindhard in the cabinet at a pressure of 484 mm. (4000 meters). Irritability, headache, nausea and loss of appetite were reported by Barcroft and his associates to be common at about 4000 meters during the

1922 expedition to the Andes. Intellectual impairment is mentioned by Barcroft. He states that on the Teneriffa expedition he was unable to solve a simple algebraic problem at an altitude of about 3500 meters. The impairment of the senses of vision and hearing, noted by Haldane, Kellas and Kennaway, is also most likely of cerebral nature. Cerebellar disturbances, such as dizziness and vertigo, are frequently reported. According to Barcroft, they appear somewhat later (at a higher altitude) than the cerebral symptoms. Zuntz and collaborators observed in 1901 on the Monte Rosa expedition that vertigo was present, even with closed eyes. Because it is reported not to occur under such circumstances in deaf-mutes, Zuntz considers vertigo to be of peripheral nature, through effects on the bulbar nerve centers.

Effect on the bulbar centres especially on pulse and respiration

According to Barcroft's experience, excitation of the bulbar nerves, like the cerebellar disturbances, appear somewhat later than the cerebral symptoms. Paul Bert has already shown that the pulse rate increased during stay in rarified air and again decreased to normal if oxygen inhalation was given. It is worth noting that whereas the increase in the pulse rate is slight during a decrease of pressure from 760 to about 470 mm., a sudden increase occurs when the pressure is lowered beyond this point. Now 470 mm. pressure corresponds to an altitude which will cause the arterial oxygen saturation of normal individuals to drop to about 80 to 85 per cent.

Paul Bert's results, therefore, indicate that a threshold exists for the action of oxygen lack on the pulse rate, similar to the threshold found for the beginning of cyanosis and for the appearance of psychic disturbances. The effect of oxygen lack on the pulse rate was later confirmed by others, for instance, Benedict and Higgins, Schneider and Sisco, and Parkinson. These investigators showed not only that the pulse rate was increased by lowered oxygen pressure in the inspired air, but also that the pulse rate dropped if air rich in oxygen (90 per cent) was inspired. Parkinson's experiments are of particular interest because care was taken to exclude any possibility of autosuggestion. The fact that increased pressure in the inspired air lowers the pulse rate is important because it supports the theory advanced by Béthe and taken up later by Lindhard, that oxygen lack acts by altering the

irritability of the nervous centers and not as an irritant, like carbon dioxide. The pulse rate seldom rises above 100. However, Haldane reports that his pulse rate increased to 136 when the barometric pressure in the air cabinet was rather suddenly lowered to 330 mm. (corresponding to an altitude of about 7000 meters). During prolonged stay in rarefied air, or at high altitudes the pulse rate drops to normal in a few days, according to Haldane, Lüscher and others. It seems most likely that the effect of oxygen lack on the pulse rate is produced through the bulbar center. A direct action on the heart, can, however, not be entirely excluded.

Of more fundamental importance for the organism as a whole, is the effect of oxygen lack on the respiration. This problem has been the object of a considerable number of investigations, and has particularly interested the schools of Bohr, Haldane, and Zuntz. As was found to be the case with the appearance of other symptoms of anoxemia, the effect on the respiration becomes manifest only when a certain degree of oxygen lack is reached. According to observations of Haldane and Poulton, a distinct increase in ventilation appears when the percentage of oxygen in the inspired air is decreased to about 12 per cent. In animal experiments, Fränkel and Geppert, and later Terray showed the threshold value to be about 10.5 per cent. Loewy demonstrated a slight increase in the ventilation even at 16 per cent oxygen in the inspiratory air, and a distinct increase was noted when the oxygen percentage was lowered to 10 per cent.

The threshold value for the appearance of changes in the respiration is, therefore, about 12 per cent oxygen in the inspiratory air. This corresponds approximately to a pressure of 450 mm. or to an altitude of about 4000 meters. The increase in the ventilation following a sudden change in the barometric pressure, or in the oxygen percentage of the inspired air, is mostly effected by an increase in the depth of the respirations, although a slight or moderate increase in the respiratory rate may also take place. If the oxygen pressure becomes very low, a decrease in the depth of respiration may ensue, so that the breathing becomes shallow and rapid. It may be recalled that an increased ventilation is much less useful if it is effected by an increased rate than if it is caused by increased depth. If the breathing becomes very shallow, it may even become less effective. After a stay of longer

TABLE 6

Rate and depth of respiration and total lung ventilation in normal resting individuals breathing air with normal oxygen tension and air with low oxygen tension

NAME	RATE PER MINUTE	DEPTH	TOTAL VENTILATION	LOCALITY	RATE PER MINUTE	DEPTH	TOTAL VENTILATION	LOCALITY	ALTITUDE OR BAROMETRIC PRESSURE
		cc.	*liters*			*cc.*	*liters*		
Kolmer	17.2	365	6.5	Berlin sea level	18	460	8.3	Monte Rosa in the Alps	4,560 meters
Caspari	14	414	5.8		12	708	8.5		
Müller	13	446	5.8		8	1,112	8.9		
Hasselbalch	9	784	7.05	Copenhagen sea level	10.7	821	8.8	Brandenburgerhaus Austria	3,290 meters
Lindhard	7	862	6.0		8.3	990	8.2		
Hasselbalch	6.7	853	5.7		7.1	990	7.0	Air Cabinet at the Finsen Institute Copenhagen	488 mm.
Lindhard	5.1	1,018	5.2		5.4	1,138	6.1		488 mm.
Maar	11.9	647	7.7		13.05	70	9.1		553 mm.

duration at high altitudes or in air cabinets with low pressure, the effect on the respiration is different. The total ventilation is only slightly increased. This is almost entirely caused by increased depth: the respiratory rate may even decrease.

This is seen in table 6 which gives the total ventilation and the respiratory rate in various individuals under such circumstances. The first five observations are from the report of Zuntz and his collaborators on an expedition to Monte Rosa. Two are from Hasselbalch's and Lindhard's expedition to Brandenburgerhaus; the last three observations in the air cabinet were made by them at the Finsen Institute. This difference in the effect on the respiration between sudden changes in the oxygen pressure and longer exposure to low oxygen pressure is important and will be discussed in greater detail later.

Besides change in the total ventilation, oxygen lack causes—as previously mentioned—change in the respiratory rhythm to periodic breathing. Haldane and collaborators observed this phenomenon on an expedition to Pike's Peak at an altitude of about 4000 meters. Haldane and Meakins found, during inspiration of air with low oxygen, periodic breathing appearing when the percentage of oxygen in the inspired air was lowered to 10 to 11 per cent.

It seems, therefore, that this symptom has approximately the same threshold value as the other symptoms previously mentioned.

Effects of oxygen lack on the metabolism

An important question is the behavior of the metabolism under conditions unfavorable for oxygen intake. One would naturally expect that the metabolism would be affected earlier and to a greater extent by oxygen lack than any other function. From the time of Paul Bert it has been generally supposed that oxygen lack of moderate degree would cause various disturbances—particularly formation of lactic acid—in the intermediary metabolism. Determinations of the total respiratory metabolism under such conditions were undertaken by Loewy in 1895. He showed that the basal metabolism, determined by the total oxygen intake, did not decrease during stay in air rarefied to such an extent that various anoxemic symptoms might be present.

In table 7 some of Loewy's results are given together with similar observations of Hasselbalch and Lindhard. It is, however, not justifiable to conclude that a quantitative normal total oxygen intake necessarily means that the oxygen supply of all the single organs of the body is normal. Only a few observations are at hand as to the oxygen intake of isolated organs during decreasing oxygen pressure. Verzar, in 1912, in Barcroft's laboratory, investigated the oxygen intake of various organs during decreased oxygen tension of the arterial blood. He found the oxygen consumption of the submaxillary gland independent of decreasing arterial oxygen tension (within certain limits).

TABLE 7

Oxygen consumption of normal individuals per minute at different oxygen tension

NAME OF INDIVIDUAL	OXYGEN CONSUMPTION PER MINUTE	BAROMETRIC PRESSURE OR ALTITUDE	LOCALITY
	cc.	*meters*	
Hasselbalch	270	756	Air cabinet at the Finsen Institute Copenhagen
	267	488	
Lindhard	234	756	
	261	488	
Loewy	185	750	Air Cabinet Berlin
	176	580	
	211	435	
	202	360	
Zuntz	228	Sea level	Berlin
	231.9	2990	Col. d' Olen, Alps
	259.2	4560	Monte Rosa, Alps

Experiments on the gastrocnemius muscle showed that the oxygen intake decreased with decreasing arterial oxygen tension. Hence he draws the conclusion that the oxygen tension in the muscle tissue is normally either zero or near zero, at any rate less than 19 mm. Experiments on the heart muscle were somewhat difficult to interpret because of inevitable variations in the work of the heart during the experiments. Verzar is, however, inclined to think that the conditions in the heart muscle are similar to those he found in the case of the gastrocnemius muscle. As to the kidneys, decreased arterial oxygen tension caused an increase in the amount of oxygen used by the organ.

In his book on the capillaries Krogh points out that experiments made in his laboratory by Gaarder also seem to indicate that the oxygen tension of certain tissues may be zero. That a decrease, first in the arterial, and consequently also in the capillary oxygen tension, necessarily must result in a decrease in the oxygen supply of these tissues where the oxygen tension normally is zero, is demonstrated by means of this formula, taken after Verzar:

$$Q = d\,(P - p)$$

where Q is the amount of oxygen which passes per unit of time, from the capillary blood to the tissues, d is a constant, P and p the oxygen tension in the capillary blood and in the tissues respectively. If P decreases Q can remain normal only if p diminishes parallel with P. This is, however, impossible if its normal value is zero.

It is interesting in this connection that muscular fatigue is one of the most constant symptoms in man during stay at altitudes great enough to produce the previously mentioned anoxemic symptoms. It seems as if the feeling of fatigue appears earlier (that is at a lower altitude) than the other anoxemic symptoms. Barcroft states that symptoms of fatigue usually appear at an altitude of 3000 meters and are always pronounced at an altitude of 4000 meters. That it is not simply due to overexertion is seen by the fact that it is present after rest and also in the morning. Whether it is of muscular or of nervous origin is not known.

Fatty degeneration as an effect of oxygen lack

It is well known that lack of oxygen may cause fatty degeneration of various tissues. It seems important that the myocardium is one of the organs which in anemia are affected earliest and most markedly. The cause of this is generally believed to be insufficient oxygen supply due to the low concentration of hemoglobin in the blood. The results of Verzar's experiments are also interesting in that respect. It is, of course, worth emphasizing that the heart muscle never is at absolute rest and therefore, even under the most favorable conditions, needs a considerable oxygen supply. Fatty degeneration is found not only in anemia (anemic anoxemia); Lewinstein has produced fatty degeneration in rabbits by keeping them under low barometric pres-

sure, and von Schrötter found marked fatty degeneration in the muscles of guinea pigs kept for three days under a pressure of about 300 mm. Hg., corresponding to an altitude of almost 8000 meters. That fatty degeneration in anemia may develop in a short time is an old clinical experience.

It is not very likely that parenchymatous fatty degeneration occurs under the conditions to which members of mountain expeditions and of air cabinet experiments have been exposed.

Effect of oxygen lack on the capillaries

Changes in the state of the capillaries are of importance for the regulation of the blood supply to various regions of the body and also for the appearance and degree of cyanosis, because the filling of the capillaries is, as pointed out by Van Slyke and myself, undoubtedly the most important modifying factor in the production of cyanosis. Krogh and his pupils have investigated the effect of oxygen lack on the capillaries in isolated organs of animals, and Liebesny and Lüscher have studied the condition of skin capillaries in man under various barometric conditions. As a whole it seems as if the state of the capillaries is little affected by oxygen lack of moderate degree.

Effect of acute and chronic lack of oxygen

It has already been mentioned that the effect on ventilation of low oxygen pressure in the inspired air is more pronounced if the change in the barometric pressure is sudden than if the change is slowly established. Similarly the effect on ventilation is much less marked if the individual has been exposed to low barometric pressure for some time. A similar difference is noted as to the behavior of the respiratory quotient during a quite acute and during a more chronic stage of anoxemia. Haldane and Poulton showed in 1907 that the respiratory quotient would reach a very high figure—between 2.75 and 3 during the initial part of an acute lack of oxygen. At the same time the ventilation was very markedly increased. This was, however, only transient; after a short time (less than half an hour) the quotient was within normal values and the ventilation not markedly increased. Such an abnormally high respiratory quotient (above 1) is met with under various conditions. It may be due to a voluntarily produced

hyperpnoea, or it may be due to a converting of carbohydrates into fat. In both instances the increase in the quotient is, however, much smaller. An increase to about 3 can be due only to such a disturbance in the acid-base equilibrium of the blood that the body for some reason or another must get rid of a considerable part of its carbon dioxide. This is further demonstrated through the behavior of the tenson of the cabon dioxide in the alveolar air in people exposed to air with low oxygen tension. In such persons the alveolar carbon dioxide tension drops down to two-thirds of its normal value in the first days of an air cabinet experiment. The fall in the tension goes almost parallel with the decrease in the barometric pressure. The final pressure is 450 mm. corresponding to an altitude of between 4000 and 4500 meters. How are these effects on the respiration to be explained? The increase in the ventilation and the low carbon dioxide tension have until the last few years been attributed to an increase in the hydrogen ion concentration of the blood, that is to say to an acidosis. By this acidosis the stimulus of the respiratory center was raised and an increased ventilation ensued. As a result of this increased ventilation the carbon dioxide was washed out first from the lungs, thereafter from the blood. Consequently the hydrogen ion concentration of the blood would decrease until a new equilibrium was established at a reaction a trifle more acid than normally. When this equilibrium was established the ventilation would diminish towards a normal, and the respiratory quotient would reach its usual value. The alveolar carbon dioxide tension naturally would remain low. This hypothetical acidosis was attributed by Haldane to production of lactic acid, which—as shown by Fletcher and Hopkins in 1907—is produced if a muscle is contracting under anaerobic conditions. However, Ryffel failed to find any lactic acid in blood or urine of individuals who were exposed to rarefied air. Later Hasselbalch and Lindhard demonstrated—as shown in figure 6—a moderate decrease in the output of ammonia in the urine during stay in an air cabinet at low barometric pressure. This has been confirmed by Haldane, Kellas, and Keenaway.

Hasselbalch and Lindhard consider the decreased NH_3 output a direct result of an injury to the liver caused by lack of oxygen. It seemed natural that such a decreased production of this very impor-

tant base might result in an acidosis. They, therefore, attributed the acidosis under such conditions to diminished production of base and not to increased acid production. However, during the last few years, Yandell Henderson and Haldane have, independently of each other, performed a long series of experiments, the results of which have led them to a new theory about the acid-base equilibrium during oxygen lack. Their theory is, to a certain extent, in accordance with the view points originally set forth by Béthe and supported by Lindhard's investigations. They assume that anoxemia causes an increased excitability of the respiratory center. The result of this is necessarily an increased ventilation. This results in a washing out of carbon dioxide. Thereby is the normal hydrogen ion concentration decreased until the establishment of a blood reaction a little more alkaline than usual is effected. In this way a new equilibrium between the respiratory stimulus and the excitability of the center is brought about. The result as to the blood reaction is a slight alkalosis. Conclusive determinations of the hydrogen ion concentration of the blood are not at hand. And it is, as Haldane remarks—not very likely that they can be produced by our present technique, because the respiratory center is much more sensitive than our best apparatus for direct determination of the reaction of the blood.

More recently doubt has been expressed by numerous investigators as to the validity of the alkalosis theory of Henderson and Haldane, Gesell, Koehler, Schneider and others. Schneider thinks that several experiences from various mountain expeditions can hardly be explained by assuming an alkalosis to be present in the blood. Be that as it may, it is at any rate beyond doubt that we must distinguish in anoxemia beween a group of symptoms caused by a disturbance of the normal equilibrium between the respiratory center and its stimuli, and another group of symptoms caused by a more permanent disturbance of the oxygen supply of certain organs. The first group of symptoms is distinct only if the change in oxygen pressure in the inspired air is very sudden, and it lasts only a short time. They constitute what we may call the first phase in anoxemia. An analogous condition is met with in changes of the body temperature, which is another important constant of the body. We have to distinguish between symptoms due to the movement of the body temperature

from one level to another, and symptoms produced after the new equilibrium has been established. And just as only sudden changes in body temperature cause sweat and chill, it is only sudden changes in the oxygen tension of the inspired air which cause distinct hyperpnoea and increased respiratory quotient.

In addition to the two phases of anoxemia already mentioned, there is a third phase, which is characterized by an acclimatization to the new conditions on the part of the body. How this acclimatization is brought about and how soon it can be established we do not know. It is present in inhabitants of high altitudes and in patients with certain heart lesions, particularly in heart lesions of congenital nature Under these conditions a distinct polycythemia is present and possibly an increase in the total amount of blood in the body. Of the anoxemic symptoms, at least cyanosis is present; about other anoxemic symptoms nothing definite is known bedause these conditions are very incompletely studied in this respect. There are a few observations at hand, which seem to indicate that this third phase may be established in a relatively short time. In the report of the Himalaya expedition of the Duke of Abruzzi, it is stated that no anoxemic symptoms were present even at an altitude of much above 4000 meters, the usual threshold value for the appearance of anoxemia.

In an expedition to Pike's Peak by Haldane and others, it was observed that the anoxemic symptoms, including even the cyanosis, disappeared in about four days. This they explained by assuming an oxygen secretion by the lung epithelium. On the other hand, experiments are on record (Hasselbalch and Lindhard, Barcroft) where no acclimatization had taken place after about one week. The problem of how the body acclimates itself to anoxemia is of great importance in human pathology, particularly in respect to certain heart, lung and blood lesions. In an acute disease, it is presumably of less or no importance, and we shall therefore not discuss it in further detail.

Résumé

We have so far in correlating the anoxemic symptoms with the incomplete oxygen saturation of the arterial blood, expressed the oxygen saturation in terms of volumes per cent or of per cent of saturation. However, this is justifiable only so far as the anoxemia is

indirectly due to the arterial oxygen deficit. To be quite correct: all the anoxemic symptoms are directly dependent not on the condition in the arteries, but exclusively on the conditions in the capillaries. And all anoxemic symptoms—except the cyanotic color—are dependent not on the amount of capillary oxygen, but on its tension, which together with the oxygen tension in the tissues determines the tissue supply of oxygen. For that reason, general anoxemia may occur in all conditions where general capillary oxygen tension is too low. The effect of localized low oxygen capillary tension has so far been studied very little. Its importance depends partly on the kind of organs affected. At present we shall confine ourselves to general anoxemia. Barcroft has subdivided anoxemia into three groups according to the secondary causes of the condition: (1) anoxic anoxemia; (2) anemic anoxemia; and (3) stagnant anoxemia, i.e., anoxemia found in stasis and slow circulation.

We have confined ourselves to a discussion of the first type of anoxic or arterial anoxemia, and so far as the experimental parts go, we have discussed only anoxemic conditions caused by low alveolar pressure (compare diagram *B* in figure 2). In principle, all forms of anoxemia are probably equal, and dependent solely on the capillary conditions. Therefore, it might seem preferable to correlate the condition of the oxygen in the capillary blood with the clinically observed anoxemic symptoms. It is, however, impossible to obtain true capillary blood (which in itself varies from arterial to venous) with our present technique. Møller and I found cutaneous blood to be saturated with oxygen to the same extent as arterial blood.

Furthermore the oxygen in the arterial blood is more directly influenced by pathological processes in the lungs than is the oxygen in the capillary blood. For these two reasons it is not only necessary but probably also advantageous to express arterial saturation in volumes per cent as the physiological basis for an analysis of anoxemic symptoms in lobar pneumonia, where it is unquestionably decreased arterial oxygen saturation which is responsible for the physiological disturbances in the capillaries, which in turn cause the symptom complex called anoxemia. In conclusion, I would again call attention to tables 1 and 5. In table 1 are given the figures for the arterial oxygen saturation in a series of patients suffering from lobar pneu-

monia. Table 5 gives the arterial oxygen saturation in a series of normal individuals at an altitude of about 4000 meters which indicates, as we have tried to show, approximately the threshold value for the appearance of anoxemic symptoms. A heavy line on table 1 indicates approximately the oxygen saturation below which we might expect anoxemia in pneumonia.

Pneumonia patients showing an arterial oxygen saturation lower than about 80 per cent are therefore under more unfavorable conditions so far as oxygen supply of the tissues goes than normal people at an altitude of about 4000 meters.

In order to make it clear to how great an extent the hemato-respiratory functions in a pneumonic patient deviate from normal conditions, I have calculated by means of a formula introduced by Van Slyke and myself the blood fraction which could pass from right to left through a defective ventricular septum of the heart before the oxygen saturation of the mixed arterial blood went below 80 per cent. This would, if the concentration of hemoglobin remained normal, be about 33 per cent of the total amount of blood traversing the heart. It is quite astonishing that there is present in one-third of the patients in table 1 an arterial oxygen unsaturation greater than that observed in normal individuals who are at an altitude of about 4000 meters or who are exposed to only 14 per cent oxygen in the inspiratory air, and greater than the arterial oxygen unsaturation caused by septal defect large enough to side track one-third of the blood from the lung circulation.

Every individual suffering from pneumonia has to struggle with an infection which in itself may be serious enough. A number of these patients have to fight under a condition of anoxemia which at best can never be favorable, and which in some instances may be the leak that sinks the ship.

II. THE EFFECT OF ANOXEMIA IN LOBAR PNEUMONIA

The problem which we shall now discuss is: To what extent can symptoms, occurring in patients with pneumonia, particularly of the lobar type be attributed to anoxemia?

Cyanosis

It was previously stated that the occurrence of the cyanotic color is dependent on the presence of reduced hemoglobin in the capillary blood. Other things being equal, the intensity of cyanotic skin color increases with the increase in concentration of reduced hemoglobin after the threshold value, about 6 to 7 volumes per cent of oxygen unsaturation, has been reached. The other anoxemic symptoms, dependent probably on the supply of oxygen to the tissues, presumably depend on the fall in the arterial oxygen tension in the capillary blood. After the threshold has been passed, these symptoms increase in intensity with decreasing oxygen tension. In normal individuals with normal concentration of hemoglobin in the blood it so happens that the threshold for the appearance of cyanosis and for the appearance of other anoxemic symptoms is passed at approximately the same time. The result of this is that the presence or absence of cyanosis indicates whether the physiological condition (i.e., a sufficiently low capillary oxygen tension) for the development of the other symptoms is present or absent. It is justifiable to assume that in most patients with pneumonia a similar parallelism between cyanosis and other anoxemic symptoms is present. The reason for this is that both abnormalities (cyanosis and other anoxemic symptoms) are the result of the same pathological conditions, namely, the decreased arterial oxygen saturation, which is in turn caused by pulmonary insufficiency. To the same extent that this parallelism is present it is fair to consider cyanosis a symptom of general anoxemia. Although this is in main true, certain exceptions must be kept in mind by the clinician who wishes to use the cyanotic color as a diagnostic guide.

Cyanosis may be present without being associated with general anoxemia in several conditions, which fall into four groups: (1) If the cyanotic color is a purely local phenomenon due to abnormally increased deoxidation of the normally saturated arterial blood. Such a local acrocyanosis is not very uncommon even in otherwise normal individuals and it can be assumed that it also may be found now and then in patients with pneumonia. (2) If the cyanosis appears at a smaller concentration of reduced hemoglobin in the capillaries than usual, due to an increased influence of the so called modifying factors: stasis, increased number of blood-filled capillaries, etc. It has been

generally assumed that a cyanotic color in pneumonia was due in the majority of cases not to respiratory but to circulatory disturbances, that is to an insufficiency of the heart. If this were true, stasis and increased peripheral deoxidation should be looked upon as the most important factors in the production of cyanosis in pneumonia. However, clinical investigations by Petren and determination of the blood gases in pneumonic patients by Stadie, le Blanc and myself have shown that a more than purely local cyanosis found in pneumonia is practically always due to respiratory disturbances, which through insufficient oxygenation give rise to abnormally decreased arterial oxygen saturation. (3) Cyanosis may be observed without the presence of other anoxemic symptoms in patients with *chronic* cyanosis. This is seen in certain congenital heart diseases and probably also in some cases of emphysema. (4) Cyanosis without anoxemia might be caused by the so called Bohr phenomenon. Bohr and his associates Hasselbalch and Krogh made in 1904 an observation which became of fundamental importance and is often referred to as "Bohr's phenomenon." They showed that increased carbon dioxide tension of the blood would cause an increased tension for the same amount of oxygen present.

If, in pneumonia, an increased hydrogen ion concentration, an acidosis, occurred at the same time as an insufficient arterial saturation it might be assumed that oxygen tension in the capillaries might be kept sufficiently high to secure an adequate oxygen supply to the tissues even if the concentration of reduced hemoglobin in the capillary blood had passed the usual threshold for the production of cyanosis. This problem, important in heart disease especially in relation to treatment with carbon dioxide inhalation seems purely theoretical so far as pneumonia goes, because Hastings, Morgan, Binger and Neill have shown that if any change takes place in the reaction of the blood in pneumonia, a very slight alkalosis is found.

In the conditions encountered in these four groups, anoxemic symptoms (except cyanosis) may be lacking either because the decrease in the tension is too small, or because it affects too few organs of importance, or because the organism in some unknown way, as in chronic cyanosis, acclimatizes itself to the abnormal conditions. In acute pneumonia patients without heart insufficiency, emphysema or poly-

cythemia, a general cyanosis indicates that the arterial oxygen saturation has decreased to 85 to 80 per cent and that the pathological conditions essential for the production of the other anoxemic symptoms may be expected to be present.

On the other hand cyanosis may be absent in cases where other anoxemic symptoms are very marked; these cases fall into two groups. First, this is seen in severe anemias. It has been shown that cyanosis does usually not develop in patients with extreme anemia because the concentration of reduced hemoglobin cannot pass the average threshold value for the development of a cyanotic skin color. And the more marked the anemia (and therefore the anoxemia) the less marked is the tendency to cyanosis, a fact which of course is of great importance for the functional diagnostic value of cyanosis in anemic conditions. However, a presence of anemia is easily recognized either by simple observations or by a determination of the hemoglobin. Secondly anoxemia without cyanosis is theoretically possible in cases of alkalosis. This is due to the Bohr effect. Due to a very low carbon dioxide tension in the blood the oxygen tension may become abnormally low even if oxygen content is normal. For that reason the capillary blood may be unable to give off sufficient oxygen to the tissues. A condition of alkalosis is rare. It has only been observed once (Binger, Hastings and Neill) in connection with pneumonia, and in this case it was due to administration of bicarbonate of soda to the patient over a considerable time. In carbon monoxide poisoning a somewhat analogous condition is met with and the skin color may be even more red than usual in spite of dangerous anoxemia. It is therefore justifiable to state that absence of cyanosis in patients with pneumonia excludes anoxemia unless the case is complicated with severe anemia, alkalosis, or carbon monoxide poisoning. *The absence or presence of cyanosis, therefore, indicates in the majority of pneumonic patients the absence or presence of the physiological conditions necessary for the development of most other anoxemic symptoms. For that reason, and because cyanosis is easily recognizable without any special apparatus, it is a symptom of great fundamental diagnostic importance in pneumonia.*

Frequency of cyanosis in pneumonia

That it is of great importance to ascertain how frequently a cyanotic color is present in pneumonia is self evident. So far relatively few

observations on this question have been published. In 1239 cases collected from the literature, Norris found cyanosis in 119 cases (10 per cent). Lyon found the symptom in 9 per cent of 109 instances of lobar pneumonia in childhood. The mortality of these cases was 4.5 per cent. In 658 cases, collected from Johns Hopkins Hospital, Chatard found, as shown in table 8, cyanosis in 33 per cent. A marked cyanosis was present in 12 per cent. The total mortality in Chatard's cases was 31 per cent. Of the cyanosed patients 43 per cent died.

TABLE 8

Frequency of cyanosis and changes in respiration in 658 patients with lobar pneumonia in the Johns Hopkins Hospital, Baltimore, Md. (Chatard)

	NUMBER		NUMBER	PER CENT	NUMBER DIED
Cyanosis	658	Mild	134	20.3	58
		Pronounced	81	12.2	35
Character of respiration	658	Dyspneic	367	55.6	140
		Strenuous	165	25.1	71
		Shallow	35	5.3	20
		Periodic	6	0.9	5
Respiratory rate	643	30	48	7.5	4
		30–40	208	32.3	31
		40–50	192	29.9	56
		>50	195	30.3	107

According to Cole cyanosis is an almost constant symptom in all severe cases of lobar pneumonia. De la Camp and Morawitz in their articles on pneumonia in Kraus and Brugsch's and in Mohr and Staehelin's handbooks respectively do not make any statements as to the frequency of cyanosis in pneumonia. It would be highly desirable to obtain accurate numerical information about the frequency of cyanosis in pneumonia and its relation to the other symptoms and to the various anatomical states of disease. If one should try to estimate the frequency of cyanosis in pneumonia from the rather incomplete information now at hand, one would say that it is present in at least 10 per cent of all cases. This figure is, however, undoubtedly too small. The mortality of the disease is approximately 20 to 25 per

cent and in almost all these cases cyanosis occurs although probably not always due directly to respiratory insufficiency. Cyanosis, usually of respiratory origin, is furthermore met with in a number of patients who recover. It is probably justifiable to assume that cyanosis (of respiratory origin) occurs in about 20 per cent of all patients with lobar pneumonia.

The clinical recognition of cyanosis

We shall not in this place enter into details concerning the clinical recognition of the presence and degree of a cyanotic color. This is best discussed in connection with bedside observations. Emphasis is required on the fact that in deciding the question of the presence or absence of the physiological conditions for development of anoxemia one can find help from the presence or absence of cyanosis only if the clinical observation of the skin color is done as carefully as the stethoscopical examinations of the lungs.

Psychic disturbances in pneumonia

As to the occurrence in pneumonia of cerebral and cerebellar anoxemic symptoms, only very little and not very precise information is yet available in the literature. Psychic disturbances of various kinds are by no means infrequently seen in patients with pneumonia. In the more severe cases such symptoms are almost always observed, often in a very pronounced degree. These symptoms are as a whole of the same rather indefinite nature as those found in experimental anoxemia. It is, therefore, at any rate not impossible that anoxemia may play a causative rôle in the development of these symptoms. On the other hand similar symptoms may be observed in other acute infectious diseases, where there is no disturbance in the oxygen supply.

The psychic disturbances encountered in pneumonia are usually of a delirious character, they may even be so pronounced that the patients leave the bed and try to jump out of the windows. And even if patients, severely ill from pneumonia, are not absolutely delirious, they are very often unreasonable and show a marked lack of judgment. Insomnia, excitability, and a certain kind of apprehension are not infrequent.

In the so called asthenic pneumonia, a name used first by Leichtenstein in 1873 to designate a certain atypical form of pneumonia, psychic disturbances are almost constantly present. A subdivision of this group is even termed the cerebral form of pneumonia. These atypical forms differ in several respects from the usual type of lobar pneumonia. Their etiology is often and their pathology almost always different. A number of cases of asthenic pneumonia therefore are not of the lobar type; many do not show a fibrinous exudate in the alveoli and most of them are probably not caused by pneumococcus. Cases of influenzal pneumonia usually belong to this asthenic form. It is of considerable interest in regard to our problem that marked cyanosis and pronounced psychic disturbances are found together in a very large number of these patients. And it is a rule that the more marked the cyanotic color the more pronounced is the psychic disturbance. It is of importance that a few observations are on record where psychic symptoms disappeared or became less marked during oxygen inhalation therapy. Meakins, Barach and Woodwell, and Stadie have described such cases where the effect of oxygen therapy suggests strongly that psychic disturbance in pneumonia patients, such as delirium, excitability and insomnia may be of anoxemic nature. It is to be hoped that if interest becomes centered on this important question, future clinical observations may bring clarity into the problem of to what extent psychic disturbances in pneumonia patients are due to anoxemia.

Pulse rate and anoxemia

In view of the fact that the increase in pulse rate observed in experimental anoxemia is rather small and usually transient, it is *a priori* not very likely that increase in pulse rate in pneumonia patients is due to anoxemia. We know, furthermore, that an increased pulse rate by no means is confined to cases showing cyanosis, which is in most cases, as we have pointed out, our clinical criterion of the presence of the physiological conditions necessary for a development of anoxemia. However, the fact that a drop in the pulse rate has been observed during oxygen treatment suggests that anoxemia may in some instances be partly responsible for the increase in pulse rate. Stadie observed for instance a drop in pulse rate from 160 to 120

following effective oxygen treatment. Whether this effect is due to an action on the bulbar center or whether it is a direct effect on the myocardium is unknown.

Peripheral circulation

Besides being of importance for the efficiency of the circulation, the behavior of the (skin) capillaries is of moment for the development of cyanosis. The few observations hitherto made on the capillaries in experimental anoxemia make it unlikely that any abnormalities which future investigations may reveal in the behavior of capillaries in patients with pneumonia can be ascribed to anoxemia.

Respiratory disturbances

The behavior of the respiration in pneumonia is of the greatest scientific and practical importance. We face at the same time a demand for an increased respiratory activity and a more or less impaired functional power of the lungs. An increase in the respiratory rate is well known to be one of the most prominent symptoms in pneumonia. If compared with pulse rate or temperature elevation the increase in respiratory rate is less marked in most other febrile diseases. In table 8 is given the frequency of respiration in 658 pneumonia patients collected by Chatard. Is this pronounced increase in respiratory rate entirely or partly an anoxemic symptom? That it is not solely due to an increased irritability of the respiratory center through oxygen lack may be concluded from the fact that an increased rate of respiration is observed also in non cyanotic patients. And in most of these cases we may assume that even if an oxygen lack is present it has not reached the intensity required for development of anoxemic respiratory symptoms. Furthermore, there is a marked and very important difference in the character of the respiratory disturbances observed in experimental anoxemia and in pneumonia.

In anoxemia produced in normal individuals by inspiration of air with low oxygen the change in respiration is, as shown in table 6, usually not very pronounced and mainly characterized by an increased depth of respiration. In pneumonia it is different. The depth of respiration is either normal or—most frequently—diminished. An increased ventilation is in almost all instances due to an increased

rate. The importance of this difference for the efficiency of the ventilation is extreme, as previously pointed out. Does that difference in the mechanism of the increased ventilation in experimental anoxemia and in pneumonia prove that the respiratory stimulus is different in the two conditions? Not necessarily, because it is natural to expect that local pathological processes present in the lungs of pneumonia patients might in some way or another modify the response of the respiratory center to the same abnormal condition in the blood (decreased oxygen tension). Several possibilities suggests themselves. In the first place one might consider the mechanical conditions within the lungs. Is it not possible that diminished lung volume and impaired mobility simply prevent pneumonia patients from increasing the respiratory depth or even from retaining the normal depth? It seems *a priori* natural. However, observations are at hand which point strongly against this explanation. We know that processes of non-pneumonic nature may encroach upon the air spaces and hamper the mobility of the lungs without preventing an increased depth of respiration under conditions where such an increase is demanded. It has furthermore been observed by Binger and Brow that the depth of respiration may increase considerably immediately after crisis, that is at a time when the consolidation is still present in the lungs. In the second place one might attribute the superficial respiration to pain which we know as a cause of superficial respiration in cases of, for instance, pleurisy, injury to the chest-wall, etc. The fact that morphine in some cases of pneumonia may increase the depth of respiration might also point to pain as the cause. However, various circumstances point against this explanation. Superficial inspiration is by no means confined to patients in whom respiration is painful (usually on account of pleurisy). Shallow breathing does not cease during sleep, which shows that the shallow breathing is at any rate involuntary. Recent studies of the action of morphine on the respiration from Richard's laboratory seem to offer another explanation of the influence of morphine on respiration sometimes seen in pneumonia patients.

Various investigations, mostly of recent years, suggest strongly another way in which local pathological processes in the lungs may influence the respiration and thereby profoundly alter the response of the respiratory mechanism to either a normal or abnormal hormone

stimulus. It might be helpful in the discussion of this problem to recall a few important points in the historic development of our conception of the maintenance of the respiratory rhythm. Three main periods are more or less distinct. Galén considered the respiratory rhythm to be an original voluntary act which has become a habit. The second period developed with nerve physiology. In this period in the first place the existence of a respiratory center in the medulla oblongata was recognized; in the second place it was shown that the activity or state of this center was influenced by various nerve impulses of reflex nature. By means of these reflexes the respiratory rhythm was maintained in its normal state or eventually altered. The most important of these reflexes was the so called Hering-Breuer reflex. According to investigations by Hering and Breuer in 1868 and by Head in 1889 the respiratory rhythm is maintained simply through the action of two centripetal nerve fibers in the lung vagus. These nerve fibers act on the respiratory center like a switchboard and initiate inspiratory and expiratory movements respectively according to the dilatation or compression of the alveoli.

The third period reached its summit when the conception of hormonal action became introduced into medicine. It is characterized by a tendency to consider respiration to be almost exclusively regulated by stimuli carried to the respiratory center by the blood. This conception can be traced as far back as 1862 to two observations, which later on were recognized to be of fundamental nature. One was Rosenthal's demonstration of the cessation of respiration after the forced deep respiration, a condition which he termed apnea. The other was Traube's observation that apnea might be produced also if hydrogen or nitrogen was inspired. This led Traube to consider carbon dioxide the stimulus to the respiration. However, it was first after the quantitative demonstration by Haldane and Priestley in 1905 of the effect of carbon dioxide on the respiration that these viewpoints gained ground and began to inspire people to further investigation of the problem.

A further support was given this theory by the demonstration, by Hasselbalch and myself in 1912, of the possibility of explaining this effect of carbon dioxide through changes in the hydrogen ion concentration of the blood, which according to our view should be considered

the adequate stimulus of the respiratory center. At the same time great progress was made in biological chemistry and it was quite natural that a tendency should prevail to ascribe most respiratory disturbances exclusively to changes in the reaction of the blood. The interest in the influence of the nervous system on the respiration was rather small in that period. However, the importance of hormonal stimulus for the activity of the respiration undoubtedly has been overrated at the expense of the stimulus of nervous origin. In the first place various pathological phenomena cannot be explained solely as a result of hormone changes, and second, several important observations point directly to a greater influence than usually assumed, on the respiratory changes from stimuli through the nerves and in particular through the vagi. Alcock and Seeman demonstrated in 1905 the presence of a demarcation current in nervi vagi even during normal respiration. Scott showed in 1908 that hormone stimulation of the respiration had a different effect according to whether the lung vagi were intact or severed. In the first case a hormone stimulation of the respiratory center increased the ventilation through increased depth and through increased rate as well. In the last case only an increased depth was seen. These experiments suggest that hormonal stimulus from the blood increases the depth whereas stimuli travelling through vagi from the lungs affect the frequency. The nerve stimuli probably cut off the depth before the distension of the lungs has reached the limit set by the mechanical structure of the chest.

Of particular interest for our conception of the influence of the vagi if pathological processes are present in the lungs are a series of investigations by Porter and Newburgh. They found that increased depth of respiration, which normally is the result of carbon dioxide inhalation, could be demonstrated in pneumonic dogs if the vagi were cut. They also showed that dogs suffering from pneumonia did not show any marked increase in the respiratory rate if the vagi were cut. In a later publication they demonstrated that the respiratory rate in pneumonic dogs would drop from 60 to about normal if the vagi were cocained. In Porter and Newburgh's experiments pneumonia of lobar type was produced by the Friedlander bacillus. The problem, why increased lung ventilation in some conditions—as for instance,

diabetic acidosis and moderate anoxemia—is effected mainly through increased depth and in other instances—as for instance in pneumonia, and in some heart cases during excessive exercise—is effected mainly through increased frequency, is undoubtedly a very complex one.

It seems almost certain that processes in the lungs of inflammatory origin may through the nervi vagi affect the respiratory center in such a way that the answer to an increased hormonal stimulus solely becomes increased frequency of respiration instead of merely increased depth. One must furthermore assume that a pathological process in the lungs may change the answer to the normal stimulus, so that the frequency of respiration may increase before the oxygen lack has passed the threshold value for increasing the excitability of the center. In this way shallow breathing might be the cause of anoxemia in pneumonia on account of the previously mentioned ineffectiveness of a respiration of small depth. A combination of impaired functional effectiveness of the ventilation probably caused mainly by a reflex from the pathological processes in the lungs and of increased hormonal stimulation of the respiratory center indirectly caused by the lowered oxygen tension in the blood may prove a very serious, at times even a disastrous condition to the patient. Through this a vicious circle may—as clearly pointed out by the English school—be established: an oxygen lack, just above the threshold, which in a normal individual increases only the depth of ventilation slightly, may, in a patient with pneumonia, increase the frequency and decrease the depth of respiration to such an extent that insufficient alveolar ventilation resulting in further decreased arterial oxygen saturation may ensue. This vicious circle the patient himself cannot, and the physician only with difficulty, break.

It has been mentioned that periodic respiration is almost constantly seen in experimental anoxemia in normal individuals. According to Chatard's clinical observations (table 8) this symptom is very rare in pneumonia. No explanation can be offered for this fact. It seems most likely that it is due to a reflex action of the respiratory center from the lungs. This symptom, clinically just as easily recognized as cyanosis, therefore becomes of very little functional diagnostic value in regard to the presence of anoxemia in pneumonia.

Oxygen metabolism in pneumonia

It may be recalled that the basal metabolism expressed in terms of oxygen intake was not diminished in experimental anoxemia. On the contrary an increase of about 20 per cent in the total intake was usually observed. It is natural to assume that an anoxemia of the same intensity does not interfere with total oxygen intake in pneumonia, not even in view of the fact that the fever increases the demand to a certain extent. However—as previously pointed out—a normal or even increased total oxygen intake, does not prove that every single organ in the body is properly supplied with oxygen. It was also pointed out that it is very likely that for instance the oxygen supply of the muscular system decreases when the capillary oxygen tension drops beyond a certain value. If in pneumonic patients similar impairment of oxygen supply to certain organs occurs it is of particular importance to two muscle groups: the respiratory muscles and the myocardium, because the demand on both is greatly increased. We shall in this lecture consider only the importance of oxygen lack to the function of the myocardium. In order to treat this obscure question intelligently it is necessary to enter into a general discussion of the function of the heart in pneumonia.

The function of the myocardium in pneumonia and its relation to anoxemia

It is generally assumed that the most direct cause of death in pneumonia is a breakdown of the circulation through heart failure. An inquiry into the literature as to the condition leading to heart failure in pneumonia yields very little and rather indefinite information. Some points may be discussed. We know that the strain on the heart muscle is increased above normal for two reasons: first, on account of the increased temperature and metabolism, and, second, on account of increased resistance in the pulmonary circulation. Whereas the first of these reasons is evident, the last needs further discussion. Lichtheim drew from his well known experiments in 1876 the conclusions that the area of the pulmonary circulation could be reduced to about one-fourth before any increased circulatory resistance occurred. Lichtheim inferred from his demonstration that the arterial blood pressure was maintained in his experiments. Rosen-

bach criticized Lichtheim's conclusions and pointed out that the arterial blood pressure in his experiments might have been kept up through compensatory vasomotor constriction and not through passage of a normal amount of blood through the lung remnants. One may add that if the pulmonary circulation had remained quantitatively normal in Lichtheim's experiments, his results could be explained by assuming that the heart by using its reserve force had overcome an increased resistance in the pulmonary circulation.

At any rate in lobar pneumonia there can be little doubt as to the occurrence of an increased resistance in the pulmonary circulation. In the first place we know that the vessels in the afflicted parts of the lungs are considerably dilated. On account of lack of tonus the dilatation can be caused by nothing else than an increased pressure; secondly, the second pulmonic sound is usually accentuated in patients unless a heart failure is present. Thirdly, there is an increased frontal area of the heart on x-ray examination.

This last phenomenon shown by Dietlen and by Levy may be a physiologic condition caused by an increased pulmonic pressure analogous to what has been found experimentally by Starling, who demonstrated a dilatation of the left heart when the arterial pressure was increased. Or it may be a pathologic condition indicating a beginning myocardial failure.

To what extent the two factors mentioned (i.e., the increased metabolism and increased pulmonary resistance) may exhaust the reserve force of the myocardium is unknown. However, experience from other pathologic conditions, where a similar increase in the metabolism or in the pulmonary blood pressure is encountered, make it unlikely that these two factors may be able to exhaust in a few days the reserve force of a normal and normally nourished myocardium.

It seems therefore necessary to assume either an anatomical or a functional injury of the myocardium in cases of pneumonia where a circulatory failure is encountered.

As to the first point not very extensive information is at hand. The few investigations hitherto published concerning the anatomical condition of the myocardium in pneumonia show that an injury of inflammatory or degenerative nature is not frequent. It is also of

importance that Romberg and collaborators were unable to detect any anatomical injury in the myocardium of animals infected with pneumococci.

Heart failure in pneumonia must therefore mainly be ascribed to a functional disturbance of the myocardium

A functional disturbance of the myocardium may be produced by two factors: toxemia or anoxemia.

Toxemia. In an infectious disease one naturally expects toxemia to play an important rôle in the breaking down of the circulation. The fact that no circulating toxin has so far been demonstrated in pneumonia can not exclude a toxic injury of the myocardium.

Newburgh and Porter have shown that the contractile power of normal myocardium diminished when transfused by blood from dogs in which pneumonia was produced by Friedländers bacillus. When the same experiment was performed on myocardium from pneumonic dogs no difference was found between the contractile power during transfusion with normal and pneumonic blood. They drew the conclusion that the myocardial tissue in some way or another had accommodated itself to the hypothetical pneumonic toxin.

Anoxemia. As to the importance of anoxemia in producing a functional impairment of the myocardium very little experimental or clinical evidence can be found. Experiences gained at high altitudes and in air cabinets show that anoxemia alone does not seem to impair the circulation during rest. On the other hand it is well known that even vigorous individuals perform only with difficulty exercise at low atmospheric pressure.

Experiments on fish embryos (Loeb) and frogs (Weizäcker) show that diminished oxygen supply may impair or even stop the contraction of the myocardium. None of these experiments are quantitative in the sense that the oxygen tension during the experiment was determined. They can therefore only give suggestions. Loeb found in certain fish embryos that the contraction rate of the heart decreased during insufficient oxygen supply. In some instances the rate decreased regularly to a certain point at which it stopped. In other instances the heart continued for hours at a slow rate. In a third group the rate fell regularly to almost zero. Weizäcker transfused

frogs' hearts with blood, the oxygen content of which was steadily diminished to about one-third of its normal value. He found little or no decrease in the work performed by the heart. However, no remarks are found in Weizäcker's experiments about the oxygen tension of the blood, which is the main factor governing the oxygen supply. And there is a possibility that carbon dioxide produced during the experiment may have increased the tension of the oxygen (Bohr's phenomenon).

Clinical experience concerning the effect on the heart functions of disturbances in the oxygen supply are very scanty and far from quantitative. Extra systoles, auricular fibrillation and heart block, not infrequently observed in pneumonia, may possibly in some instances be caused by anoxemia. In other and probably more instances the cause may be toxemia. Occurrence of heart block in pneumonia is of particular interest because it is experimentally shown that heart block may be caused by oxygen lack.

In 1915 I observed a transient attack of heart block in a cyanotic patient with pneumonia. Attacks of heart block are most frequently seen in the convalescent stage, i.e., at a period where no anoxemia is present. Attention has been called to the dilatation of the heart found by Dietlen and by Levy, and it was mentioned that such a dilatation may indicate a functional disturbance of the myocardium.

In spite of the lack of experimental and clinical information about the functional condition of the myocardium in pneumonia it seems justifiable to expect that insufficient oxygen supply of the myocardium may in some instances cause a breakdown of the heart, especially if it is injured by toxins and hampered by increased resistance.

The adaptation of the pneumonic organism to anoxemia

We have divided the effects of the experimentally produced anoxemia into three groups: first, a series of symptoms indicating the endeavour of the organism to adapt itself to the new condition. This phase was analogous to what happens when an organism is trying to reach an equilibrium at a temperature higher than the normal. The second group of symptoms represented the symptoms which prevailed after this equilibrium had been reached. The third phase represented the slow accommodation of the organism to a

chronic anoxemia seen in certain heart diseases or during long lasting exposure to low barometric pressure.

We have so far in our discussion of anoxemia in pneumonia mainly discussed the second phase. However, the question about the first phase is an important problem in pneumonia for the reason that it is

TABLE 9

Oxygen saturation, carbon dioxide tension of arterial blood, and body temperature in 8 patients with lobar pneumonia at Rockefeller Hospital, (Binger, Hastings, Morgan and Neill, personal communication)

NUMBER	OXYGEN SATURATION OF ARTERIAL BLOOD	TENSION OF CARBON DIOXIDE IN ARTERIAL BLOOD	BODY TEMPERATURE
	per cent	*mm.* Hg	
3		36.9	38.7
		45.6	37.7
4		37.5	39.6
	84	36.5	38.8
		40.3	37.3
5	68.2	44.7	37.8
	97.2	34.8	39.6
8	64.2	42.3	37.4
	90.5	35.1	39.6
9	94.4	40.3	39.4
	97.0	44.6	37.3
13		34.8	40.2
	89.7	40.7	37.3
14	91.8	40.3	37.4
	96.2	33.3	39.7
15	68	42.8	39.8
	81	40.3	40.2
	84	37.3	37.3

mainly by means of the respiratory function that an organism reaches a new equilibrium. One should naturally expect that the conditions in a pneumonic patient are unfavorable in that respect. The question is: Do pneumonic patients suffering from anoxemia wash out part of

the carbon dioxide of the body in order to lower the hydrogen ion concentration and thereby protect the respiratory center? A few determinations of the carbon dioxide content of the blood in pneumonia are at hand. As a whole the values are below normal. But as the important factor is the carbon-dioxide tension and not the content these determinations are of only suggestive value because the content and the tension of this gas do not necessarily run parallel. Reliable determinations of the carbon dioxide tension of the alveolar air in pneumonia are naturally not at hand.

In table 9 are given the results of a series of determinations of the carbon dioxide tension of the blood in pneumonia done in 1923 in the Rockefeller Hospital.

In most cases the arterial oxygen unsaturation was also determined. As a whole no considerable decrease in the carbon dioxide tension is found. At any rate the decrease is much less than that found in normal people with experimental anoxemia of the same degree. Furthermore no parallelism can be demonstrated between the—moderate—decrease in the carbon dioxide tension and the degree of arterial oxygen unsaturation. The decrease in the carbon dioxide tension of the blood is therefore not to be looked upon as a washing out of the carbon dioxide on account of anoxemia.

There seems to be no doubt that the low carbon dioxide tension is related directly or indirectly to variations in the body temperature, an important fact demonstrated originally by Fridericia and Olsen in the alveolar air of patients with febrile diseases.

Determination of the hydrogen ion concentration of the blood in the patients in table 9 showed values[3] either normal or slightly alkaline. These determinations show either that pneumonic patients with anoxemia are unable to lower the carbon dioxide tension of the blood or that such patients for some unknown reason do not need this regulation which, as previously mentioned, is present in normal individuals exposed to anoxemia. In the first case one should expect a permanent overexcitation of the respiratory centre the excitability of which is increased through the anoxemia. In the second case one must assume that the kidneys or the liver have taken over that part of the regulation which the diseased lungs cannot perform.

[3] Not given in table 9.

The third phase, the effect of chronic anoxemia on the body, will not be discussed here because it is undoubtedly of little or no importance in an acute disease as lobar pneumonia.

Oxygen treatment of pneumonic patients

The results of oxygen treatment of pneumonic patients will be mentioned only so far as they are of importance for the elucidation of anoxemic symptoms. During the last four or five years about 40 cases have been published in which the treatment, the blood gas determinations, and the clinical description are such that the results may throw light on the question of the anoxemic symptoms in pneumonic patients.

In about 60 per cent of the cases the oxygen content of the arterial blood has been increased to normal values. This very important fact shows that only in 40 per cent of the patients is the abnormally low arterial oxygen saturation caused by perfusion of consolidated lung areas inaccessible to air.

In most instances cyanotic color has disappeared when the abnormal oxygen unsaturation was abolished, showing as previously mentioned that cyanosis in pneumonia usually is of respiratory origin. In a case published by Woodwell and Barach a moderate acrocyanosis was present even after the oxygen content of the arterial blood had become normal. In this case the cyanosis must be attributed to increased peripheral deoxidation of the capillary blood through stasis. Also other symptoms of circulatory failure were present.

In several patients where pronounced cerebral symptoms were observed, Meakins, Barach and Woodwell, and Stadie found that after oxygen therapy these symptoms either disappeared entirely or became less. The patients became less delirious and quieter and the sleeplessness disappeared. In some instances the effect of oxygen treatment on the psychic disturbances has been remarkably striking. The pulse rate is usually not much influenced by oxygen therapy. In a few patients slowing of the pulse rate does occur and in one of Stadie's cases a drop from 160 to 120 was observed when the anoxemia was abolished. Whether or not this effect may be ascribed to a better oxygen supply of the myocardium we do not know.

The effect of oxygen therapy on the respiratory rate is of importance for our conception of the nature and pathogenesis of the dyspnoea in pneumonic patients. In most cases successful oxygen therapy has been followed by only a small drop in the respiratory rate, an observation which is in accordance with our conclusions in the chapter on the respiration in pneumonia. It is clear that if an increased sensitivity of the respiratory centre, caused by decreased blood oxygen tension, were the cause of the dyspnoea, this condition should be alleviated or abolished when the blood oxygen became normal. The results of the oxygen therapy gained so far, therefore, support the conception that the main cause of the dyspnoea in pneumonic patients is a reflex from the local processes in the lungs.

The results of oxygen therapy do not help us to decide whether or not a restoration of normal arterial oxygen unsaturation has any effect on the function of the myocardium. The fact that we do not possess any suitable functional test for the heart makes it improbable that we shall gain any information before a very large amount of material is collected or before experiments on isolated hearts are performed.

The material is also too small and clinically too heterogeneous to yield any information about the influence of oxygen therapy on the course of the disease.

Résumé

The term anoxemia may at present appear strange and unreal. In a few years however it will be as familiar as uremia. This last term is in several respects similar to anoxemia, but much more obscure, in spite of the fact that for a long time it has been a part of the pathologic system. Both terms comprise groups of symptoms and not etiologic entities, and both are a result of severe impairment of the function of important organs. But whereas uremia in the majority of instances indicates a desperate terminal condition caused by irreversible anatomical disturbances, anoxemia in pneumonic patients is caused by a disease where the possibility of complete recovery is present. It is therefore of the greatest importance to treat this symptom as soon and energetically and, above all, as scientifically as possible.

Summary

The main points developed above are the following:

1. In 10 to 20 per cent of patients with lobar pneumonia disturbances in the respiratory functions of the lungs cause an abnormally low oxygen saturation of the arterial blood. This in turn gives rise to a decrease in the amount and tension of the oxygen of the capillary blood.

2. Through physiological and pathological investigations it is demonstrated that when a decrease in the arterial oxygen content (and consequently also in the oxygen content and tension of the capillary blood) exceeds a threshold of 15 to 20 per cent a group of pathologic symptoms appears in normal individuals. The main symptoms are: Cyanosis, mental disturbances, abnormal irritation of the nervous centres in the medulla, particularly of the respiratory centre, probably an impairment of the function of the muscular system, particularly of the myocardium.

The term anoxemia is in most instances used to indicate the condition characterized by these symptoms. In other instances anoxemia simply means the underlying pathologic condition, namely the incomplete oxygen content of the blood.

3. An attempt has been made to show by analyses of different facts published in the literature that the various anoxemic symptoms in the main appear at the same threshold level of arterial oxygen unsaturation. The presence or absence of a cyanosis may therefore in most instances serve as a clinically useful criterion for the presence or absence of an arterial oxygen deficit extensive enough to give rise to other less characteristic anoxemic symptoms.

4. In pneumonia similar symptoms are frequent. A deeper analysis of these symptoms shows first that anoxemia probably plays a part in their development, secondly that anoxemia is to be looked upon only as one of the causes of these symptoms. To what extent some of the most important functional disturbances in pneumonia, namely the dyspnoea and heart failure, are caused by anoxemia and to what extent by other factors is not known.

5. In a considerable number of pneumonic patients with abnormally low arterial oxygen saturation treatment with oxygen inhalation has

caused the abnormal oxygen unsaturation to disappear. At the same time the anoxemic symptoms, especially the cyanosis, have either disappeared or decreased in intensity.

In the British Physiological Society Barcroft, in 1920, in a lecture on anoxemia, said that "The physiology of today is the medicine of tomorrow." I consider this right only if between "today and tomorrow" intense and conscientious work is done to reconcile the synthetic researches of the physiologist with the analytic investigations performed at the bedside by the clinician.

To do that not only in respect to physiology but also in respect to the other biological sciences is the main purpose of the theoretical medicine. And the delineation of the problem is, to my mind, the main purpose of the teaching of theoretical medicine.

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IMMUNIZATION AGAINST PNEUMONIA

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The high incidence rate of lobar pneumonia is a well known fact and remains a constant source of anxiety to the public health officer. Obviously one of the most important sanitary problems of the day, the prevention of pneumonia is contingent on either the elimination of the virulent pneumococcus or the production of some form of artificial immunity against it. At the present time the former procedure is entirely impractical. Pneumonia does not lend itself readily to control by ordinary hygienic or sanitary measures. The high incidence of pneumonia in cities is evidence that the pneumococcus thrives best when people live under crowded conditions. Infection appears to be transmitted by direct or indirect contact, in many instances by the droplet method. With the present tendency toward urbanization of our population there seems to be less and less hope of controlling pneumonia and other respiratory diseases by any of the hygienic measures now in vogue.

From these considerations it would appear that for the present at least the only hope of preventing pneumonia lies in the adoption of some method of artificial immunization. The simplest way to prevent pneumonia would be to prevent the common cold; for it is well recognized that pneumonia in man is usually secondary to a cold or a sore throat. Unfortunately there is as yet no universally satisfactory method of preventing colds or sore throats, consequently until such a method is forthcoming, the attack upon pneumonia must be centred upon that disease itself.

In view of the established value of vaccination against typhoid fever, it is really surprising that efforts to prevent pneumonia by means of vaccine were not undertaken earlier. As a matter of fact it has been only in the last few years that any serious effort has been made along this line.

Lobar pneumonia is an acute infectious disease, caused, in the great majority of cases, by the pneumococcus. Approximately 95 per cent of all cases of true lobar pneumonia are of pneumococcal origin. The streptococcus and Friedlander's bacillus are responsible for the few remaining cases. In this article our attention will be confined to a consideration of pneumococcus immunity and its bearing on the prevention of pneumococcus pneumonia.

IMMUNITY FOLLOWING LOBAR PNEUMONIA

It will not be out of place at this point to refer briefly to the four biological types of pneumococcus as first differentiated by Dochez and Gillespie (1). Morphologically and culturally these four types resemble one another closely but they display sharp and specific differences in respect to agglutinins, precipitins, and protective bodies. It has, of course, been necessary to take these facts into consideration in an attempt to vaccinate against pneumococcus infection.

When an animal or man survives an attack of pneumococcus pneumonia, a high degree of immunity against the homologous type of pneumococcus is readily demonstrable. The crisis itself is a striking expression of this immunity. Furthermore, Dochez (2) has shown that the serum of patients convalescing from pneumonia contains protective substances against the homologous type of pneumococcus, and Blake (3) has demonstrated precipitins in the serum of cases of pneumonia that terminate favorably. In addition to these clinical studies, accurate information on the subject of immunity following pneumonia has been obtained from experimental work on animals. In some studies on experimental pneumonia conducted by Cecil and Blake (4) in 1920, these authors showed that in monkeys an attack of pneumococcus Type I pneumonia protects the animal completely against a second infection of the same type. An attack of pneumococcus Type I pneumonia gave little if any protection against pneumococcus Type II pneumonia. On the other hand, there appeared to be some cross-protection against pneumococcus Type III.

During the last five years we have had opportunity to follow a number of patients through several attacks of pneumonia and in every instance recurring attacks have differed in type from the initial type.

The one exception appears to be in the case of pneumococcus Type III infections. There are records of several patients who have had repeated attacks of pneumococcus type III pneumonia. These attacks, however, rarely come oftener than once a year.

If one can make any inferences from these observations they would be that: (*a*) An attack of lobar pneumonia is followed by a high grade of immunity against the type of pneumococcus that excited the attack. (*b*) This immunity is not permanent but probably lasts from six months to one year. (*c*) There is a small amount of cross-immunity against other types of pneumococcus. This, however is probably a variable and uncertain factor.

ACTIVE IMMUNIZATION AGAINST THE PNEUMOCOCCUS

The earlier investigators were much interested in pneumococcus immunity. A. Frankel (5) made the important observation that rabbits inoculated with living, virulent pneumococci showed a high immunity if they recovered from the infection. G. and F. Klemperer (6) induced a high degree of active immunity in rabbits against pneumococcus in several different ways. They inoculated animals with heated pneumonic sputum, with pus from a pneumococcus empyema, and with cultures of pneumococcus which had been heated for one hour at 60°C. Emmerick (7) injected rabbits with cultures which produced marked emaciation in the rabbits but did not kill them. By this method he produced a very high immunity, the animals withstanding 20 to 30 cc. of highly virulent culture intravenously. These and other investigators have shown that an adequate immunity against pneumococcus infection can be developed in animals. Neufeld (8) produced a high immunity in rabbits by subcutaneous and intravenous injections of killed pneumococci. He found, however, that it was necessary to use a virulent culture. Levy and Aoki (9) have immunized animals with pneumococci killed by phenol and also with sensitized pneumococci.

Lister (10) found that specific agglutinins and opsonins could be produced in rabbits very easily by small doses of pneumococcus vaccine inoculated intravenously, and also, but with greater difficulty, if inoculated subcutaneously. Such rabbits thus immunized were also able to resist an attack by virulent pneumococci when the latter

were inoculated in doses that would invariably kill a normal unvaccinated rabbit.

Some interesting studies on pneumococcus immunity were carried out in 1917 by Solis-Cohen and Heist. (11) Their observations were made on the whole blood of rabbits inoculated with killed pneumococci. According to these experiments the whole blood of animals immunized against the pneumococcus possesses bactericidal power against pneumococci which is not possessed by the serum or defibrinated blood. The bactericidal power thus induced was specific as regards type of pneumococcus.

In addition to the immune bodies just referred to, the serum of an animal immunized against pneumococcus contains the so-called "protective substance" which has the power of protecting mice and other laboratory animals against lethal doses of virulent pneumococcus. These various immune substances rarely make their appearance in the blood of the immunized animal before the seventh or eighth day after the first injection of vaccine.

Larsen and Nelson (12) however, have recently found that if pneumococci are first treated with sodium ricinoleate, immune substances make their appearance much earlier in the blood of the vaccinated animal than when treated with ordinary vaccine.

It is clear from this brief review of the literature that the pneumococcus differs in no way from other pathogenic bacteria in its capacity to stimulate active immunity in animals.

The serum of animals vaccinated against pneumococcus not only develops bodies which provide the animal with an *active immunity* against the homologous type of pneumococcus, but the serum from these immunized animals is also capable of inducing *passive immunity* in normal unvaccinated animals against a fatal infection and even of curing them after infection has taken place, provided injection of the serum is not too long delayed. This fact forms the basis for the modern serum treatment of lobar pneumonia in man.

ACTIVE IMMUNIZATION AGAINST EXPERIMENTAL PNEUMONIA

While so many careful studies were being made of pneumococcus immunity, it was strange that little or no effort was being directed toward the study of active immunity against pneumonia itself. The

reason for this failure was no doubt to be found in the difficulty which earlier investigators had in producing a satisfactory experimental pneumonia in animals.

In 1904 (13), Wadsworth undertook to produce an active immunity against experimental pneumonia in rabbits. Wadsworth injected rabbits intratracheally with virulent pneumococci and thereby excited a patchy form of pneumonia. He then vaccinated normal rabbits with a saline suspension of pneumococci dissolved in rabbit bile. The immunized rabbits were subsequently injected intratracheally with 1 cc. of an extremely virulent culture of pneumococcus. Of the 11 immunized animals none died, but a few were seriously ill for twenty-four to thirty-six hours. When killed the vaccinated rabbits showed areas of diffuse consolidation involving considerable parts of the lung. Of the 5 control rabbits, 3 died without lung lesions; the 2 others lived a few days longer and showed at autopsy small areas of consolidation in the lungs. It is evident from these experiments that Wadsworth produced a partial immunity in rabbits against pneumococcus infection. His infecting dose, however, was too large for the amount of immunity produced.

In 1920 Blake and Cecil (14) perfected a technique for producing experimental lobar pneumonia in monkeys. By injecting these animals intratracheally with minute doses of virulent pneumococci they were able to produce a typical lobar pneumonia differing in no respect clinically or pathologically from lobar pneumonia in man.

Cecil and Blake (15) then undertook to study the effect of prophylactic vaccination against experimental pneumococcus pneumonia in monkeys. In these experiments small doses of pneumococcus lipovaccine were used and each monkey received one inoculation subcutaneously. By this method of vaccination partial immunity against the homologous type was established, but not enough to prevent mild attacks of pneumonia. In a later study on monkeys Cecil and Blake found that the subcutaneous injection of a small dose of living, virulent pneumococci produced a high degree of active immunity sufficient to protect animals completely against experimental pneumonia of the homologous type. Living cultures also stimulated a small amount of cross immunity against other types of pneumonia, which, however, varied considerably with individual animals. Vaccination

with living, virulent pneumococci caused severe, at times fatal, reactions, while in other instances the reactions were very mild.

It seemed quite possible that failure to obtain immunity with killed cultures of pneumococcus was due largely to inadequate dosage. The writer, therefore, in collaboration with G. I. Steffen (16), continued the study of active immunity against pneumococcus pneumonia in monkeys, and found that the subcutaneous inoculation of monkeys with three large doses of pneumococcus Type I vaccine conferred upon them a complete immunity against experimental pneumococcus Type I pneumonia. Cecil and Steffen also found that the intravenous inoculation of small doses of pneumococcus Type I vaccine conferred complete immunity against the homologous type of pneumonia. In subsequent experiments (17) they found that as in the case of pneumococcus Type I, three subcutaneous injections of pneumococcus Type II vaccine would confer on monkeys a complete immunity against pneumococcus Type II pneumonia. A similar protection could be bestowed on monkeys against pneumococcus Type IV pneumonia by three subcutaneous injections of a vaccine prepared from the same strain of pneumococcus. The subcutaneous injection of monkeys with three doses of pneumococcus Type III vaccine conferred complete immunity against this type in only 50 per cent of cases (4 out of 8 monkeys vaccinated). In view of the difficulties associated with the vaccination of horses against pneumococcus Type III, the failure to obtain immunity against pneumococcus Type III in 100 per cent of monkeys was not surprising.

INTRATRACHEAL VACCINATION AGAINST PNEUMONIA

Several years ago it occurred to the writer that it might be feasible to establish an immunity against pneumonia by injecting pneumococcus vaccine directly into the trachea. Such a procedure seemed entirely rational in consideration of the fact that in lobar pneumonia infection takes place through the trachea, and furthermore, that in the early stages pneumonia is a peribronchial infection. In the first series of experiments carried out by Cecil and Steffen (18), ordinary pneumococcus vaccine was injected directly into the trachea of monkeys with a hypodermic syringe. Each monkey received three injections of vaccine at intervals of five to seven days. It was

found that monkeys could be readily immunized against the various types of pneumococcus by intratracheal injections of pneumococcus vaccine. Indeed, the successful immunization of monkeys with three small intratracheal doses of vaccine afforded some evidence that pneumococcus immunity might be more readily induced by the intratracheal route than by the subcutaneous route. In this connection it is interesting to note that monkeys vaccinated subcutaneously or intravenously against pneumococcus usually showed immune bodies in the circulating blood, while the monkeys vaccinated by the intratracheal route failed to show circulating antibodies. This suggested that the immunity induced by the intratracheal injection of vaccine was in great part a cellular immunity.

Cecil and Steffen also attempted to immunize monkeys against pneumonia by spraying them with pneumococcus vaccine. Complete immunity against pneumonia was not obtained by this method of immunization. The failure to obtain immunity by means of a spray may have been due to technical difficulties, but in this connection it will be appropriate to discuss the work of Stillman.

Stillman (19) exposed mice to an atmosphere containing large numbers of virulent pneumococci. He found that a high degree of active immunity could be produced in mice following repeated inhalations of living pneumococci, but a much less marked increase in resistance was afforded animals exposed to a spray of dead organisms. In order to explain this acquired protection Stillman assumed that the inhaled pneumococci actually gained access to the body tissue; that is, they were not only implanted on the mucosa of the lower respiratory tract but actually penetrated the respiratory epithelium. In sharp contrast to the favorable results obtained by the inhalation of living pneumococci, Stillman obtained only a slight immunity in mice by the repeated inhalation of killed organisms. An interesting fact brought out by Stillman was that mice which had been immunized by inhalations of living or killed pneumococci, could be deprived of this immunity by subjecting them to alcoholic intoxication.

ACTIVE IMMUNIZATION AGAINST PNEUMONIA IN MAN

Prior to 1911 no serious attempt had been made to vaccinate human beings against lobar pneumonia by means of pneumococcus vaccine.

In 1911, however, the mortality from lobar pneumonia among the tropical natives in the South African Gold Mines became so high that the gold mining industry secured the services of Sir Almroth Wright to investigate the disease with a view to devising some method of diminishing the death rate.

The investigations conducted by Wright and his co-workers extended from October 1911 throughout 1912. Wright (20) was chiefly interested in detecting differences in the serum of inoculated and uninoculated persons, particularly in respect to the opsonic index. Wright was seeking some standard by which he could correlate the amount of immunity with the dose of pneumococcus vaccine and thus determine the most satisfactory prophylactic method of vaccination against pneumonia. Wright's studies of the opsonic index after injections of pneumococcus vaccine failed to disclose any definite information. Wright also found that the agglutination reactions were very irregular with the vast majority of strains of the pneumococcus.

Wright next had recourse to inoculating several thousand miners with killed broth culture vaccine, setting aside control groups of natives for comparison. The incidence of pneumonia among the vaccinated men six months to one year after inoculation was recorded and a similar record kept of the incidence of pneumonia among the unvaccinated miners. These control groups, however, were not clearly defined and led to great difficulty in estimating the results of the various experimental inoculations.

Wright's method of inoculation consisted at this time of the subcutaneous administration of one dose of pneumococcus vaccine containing 4 to 50 million killed bacteria. Wright himself was convinced from his study that the incidence of pneumonia was considerably reduced during the first three months following inoculation. Later reports, however, failed to establish the efficacy of his method of vaccination against pneumonia. His failure was probably due to two things: First, at the time his experiment was conducted, the various types of pneumococcus has not been distinguished; second, the doses of vaccine employed were much too small.

In 1913 Dochez and Gillespie published their classification of pneumococci and Lister (21) independently reported shortly afterwards a similar classification of the pneumococci in South Africa. Lister

(22) then undertook an experimental study of prophylactic inoculation against the various types of pneumococci in animals and man. He demonstrated that immunity could be produced in man against at least certain ones of these types either by subcutaneous or intravenous injection, more readily by the latter. He found that subcutaneous inoculation of 40 billion cocci of the strains he employed caused little if any toxic reaction in the guinea pig, rabbit or man, and that even intravenous inoculations of several billion in the rabbit or in man gave rise to but slight toxic reaction.

Experiments carried out by Lister at the South African Institute for Medical Research also proved beyond doubt that the blood serum of natives who had been inoculated with suitable doses of pneumococcus vaccine contained agglutinins and opsonins in substantial amounts. The number of organisms necessary to produce these changes was, however, far in excess of anything previously attempted, but, as in the case of rabbits, fewer organisms were necessary when the intravenous route was used than when the subcutaneous route was employed.

In view of these favorable preliminary experiments Lister next undertook the prophylactic inoculation of large groups of miners against pneumonia. Lister's nomenclature was somewhat different from that of Dochez and Gillespie. He found that approximately 70 per cent of all cases of lobar pneumonia in South Africa were caused by infection with either group A, B or C, groups B and C corresponding to Types II and I respectively in Dochez and Gillespie's classification. In Lister's first experiment 10,866 recruits were each inoculated three times with a vaccine containing equal parts of pneumococcus groups A, B and C. In some instances Lister vaccinated natives intravenously, but later he adopted the subcutaneous method, giving each volunteer three subcutaneous injections consisting of 6 billion cocci of each group (a total of 18 billion). Subsequently he greatly reduced this dosage and gave three subcutaneous inoculations each injection consisting of 2 billion of each type. Pneumococcus vaccine as at present prepared at the South African Institute for Medical Research contains 8 billion pneumococci per cubic centimeter comprising 8 different pneumococcus groups.

The results of this experiment were very satisfactory. Not a single case of lobar pneumonia due to groups A, B or C occurred among the

vaccinated troops. Eighty-two cases due to other types occurred among the vaccinated troops. During this time the case mortality among unvaccinated troops was 34.7 per cent. Lister contends that this fact, namely, the alteration of a relative group prevalence by means of specific group inoculation, is a more critical test of the efficacy of pneumonia prophylaxis than the simultaneous comparison of pneumonia rates in inoculated and uninoculated (control) groups when the comparison is based upon the erroneous assumption that all cases of disease due to the pneumococcus are bacteriologically indistinguishable. He emphasizes the probability that the protection of a considerable part of the community by inoculation lessens the number of carriers, and perhaps the virulence of the strains found in the community, and, hence, confers a definite benefit upon the uninoculated group which would affect the use of this group as controls in a statistical sense. Lister reported no unpleasant effects from the vaccine.

Following this experiment on the Crown Mine, Lister carried out similar experiments at the Premier Diamond Mine in the Transvaal and at the DeBeers Diamond Mine at Kimberly. At the Premier Mine the death rate fell during the experimental period to about 1 per 1000 per annum, while at Kimberly in the DeBeers Mines the mortality from pneumonia was quickly reduced by more than 50 per cent. These experiments of Lister's were carried out during 1916 and 1917. During 1918 Lister arranged to inoculate all new native mine recruits with pneumococcus vaccine, three doses to be given at seven days interval. In Lister's (23) last report of March, 1924, he reviews the results obtained during the six years previous. During this period there was a drop in the pneumonia death rate from 5 per 1000 in 1916 to 1.68 per 1000 in 1922. There was a slight increase in 1923 and 1924, but the figure remains in the neighborhood of 2 per 1000 of population. According to Lister, infections due to the three great groups of pneumococci (A, B and C) are now relatively rare in South Africa and the great majority of the lobar pneumonias that do occur are due to strains other than those of the A, B and C groups.

In South Africa the question has been raised, whether the improvement in the general hygiene at the Mines has not been the chief factor in the decreased pneumonia mortality. Lister points out, however, that the Premier Diamond Mine was about the worst mine in

South Africa as regards mortality for pneumonia, yet it was for the most part an open mine with abundant ventilation.

In the winter of 1917–1918 lobar pneumonia became quite prevalent among the recruits who were in training at Camp Upton, N. Y. Bacteriological study of the cases showed that a high percentage were due to the pneumococcus. In consideration of the favorable results achieved by Lister in South Africa, it seemed desirable to the writer to test the prophylactic value of pneumococcus vaccine on Camp Upton troops. In collaboration with J. H. Austin (24) of the Rockefeller Institute an extensive field experiment was undertaken with a vaccine containing equal parts of pneumococcus Types I, II and III. Vaccine against Type IV was obviously not feasible on account of its many varieties. The vaccine was first tested on rabbits and on 42 adult volunteers. It was found that an ordinary pneumococcus vaccine prepared from glucose broth cultures would stimulate agglutinins and protective substances within eight days after the first injection of vaccine. Generally speaking, the larger the dosage of vaccine the better the response both in agglutinins and in protective bodies. On the basis of these preliminary experiments, we adopted for the large experiment a total dosage of 6 to 9 billion cocci of each type administered in 3 or 4 doses at weekly intervals.

Altogether, 12,519 men were vaccinated—about 40 per cent of the mean strength of the command. A great majority of these received three or four inoculations. Some, however, had only one or two. The vaccine was given at five- to seven-day intervals, except in one organization where unavoidable circumstances necessitated a lapse of twenty days between the first and second inoculations. In most cases the vaccine was administered in the arm.

Reactions

The constitutional reactions to the injections of pneumococcus vaccine were usually negligible. Out of the entire number vaccinated, only 25 men were sufficiently ill to remain in quarters or in the hospital for a short time. This number would probably have been larger if the vaccination had been compulsory. As it was, those who were upset by the first or second inoculation were usually not given the third or fourth. The impression prevailed among the regimental

surgeons that the reactions to pneumococcus vaccination were milder than those to typhoid vaccination. The troops, however, were in better physical condition when they received the pneumococcus vaccine than when the typhoid inoculations were given. In those who reacted severely the symptoms simulated an attack of influenza. The patient complained of general malaise, chilly sensations, fever and muscular pains. In addition, a certain number of those who reacted severely had symptoms referable to the upper respiratory tract, such as coryza, sore throat, cough, and pain in the chest. A number of those who were suffering from infections of the air passages at the time of inoculation, claimed that their symptoms were more marked after receiving the injection. Constitutional reactions to pneumococcus vaccination apparently develop more slowly than with typhoid inoculation, sometimes not appearing until twenty-four hours after the injection.

The local reaction to pneumococcus vaccination differs little, as a rule, from that to typhoid vaccination. At the point of inoculation an area of tenderness and induration develops, usually about 5 to 10 cm. in diameter. The area of induration is nearly always oblong and extends down the arm below the point of inoculation. The axillary glands are sometimes swollen and tender. The tenderness and swelling at the point of inoculation rapidly decrease, however, and at the end of three or four days have usually disappeared.

An unexpected and somewhat troublesome complication arose in connection with the vaccination which at first gave some concern; this was the development of a certain number of small infiltrations at the site of inoculation. They developed slowly, rarely coming to the stage of fluctuation before the sixth or seventh day after inoculation. They were usually 2 or 3 cm. in diameter, and in only one instance extended down deeper than the subcutaneous tissue. At first they were looked upon as the result of careless technique, but repeated cultures showed them to be invariably sterile. Some of them developed after the first inoculation, but a greater number followed the larger doses. In a few cases an infiltration developed with each inoculation given. This would have probably happened more frequently had the vaccination not been discontinued in those who developed the condition after the first or second dose. At first the regimental sur-

geons made an incision, but later it was found that the infiltrations would progress favorably if left alone. Altogether, 152 men developed the lesion (1 in every 82). The infiltrations were tender and painful in the early stage of their development but later became cold and painless.

Results of vaccination

The vaccination of the troops was begun on February 4, 1918. The Division was transferred from Camp Upton about April 15, 1918. The following figures are based on the period extending from February 4 to April 15, about ten weeks. The number of troops vaccinated was 12,519. The number of unvaccinated was approximately 19,481. The latter figure varied, of course, from day to day as new men came and others departed. The vaccinated men were in stable organizations where the personnel underwent little change.

During the ten weeks subsequent to vaccination no cases of pneumonia due to the three fixed types of pneumococcus occurred among the troops who had received two or more injections of vaccine. One individual developed pneumococcus Type I pneumonia twenty-four hours after the first injection of vaccine, that is, before any protection could have been induced by the vaccine. In the control group of approximately 20,000 unvaccinated men, there were 26 cases of pneumococcus Types I, II and III pneumonia during the same period. Strangely enough, the incidence of pneumococcus Type IV pneumonia and of streptococcus pneumonia was also much lower among the vaccinated troops than among the unvaccinated. There were 9 Type IV pneumonias and 7 streptococcus pneumonias among the vaccinated troops, whereas, among the unvaccinated men there were 33 type IV pneumonias and 106 streptococcus pneumonias. The case mortality rate among the vaccinated was only 11.7 per cent, while that for the unvaccinated was 28 per cent.

The results obtained by Cecil and Austin at Camp Upton were so promising that further tests with pneumococcus vaccine were undertaken the following winter.

Cecil and Vaughan (25) conducted a second field experiment with pneumococcus vaccine at Camp Wheeler, Georgia. On this occasion 13,460 men, about 80 per cent of the entire camp strength, were

vaccinated against pneumonia with a pneumococcus vaccine containing 10 billion each of pneumococcus Types I, II and III in each cubic centimeter of vaccine. In this experiment, however, the pneumococci were suspended in cottonseed oil instead of the usual salt solution. Each soldier received a single injection subcutaneously. The dose was 1 cc. of the lipovaccine, equivalent to 30 billion pneumococci. Conditions at Camp Wheeler were not nearly so favorable for testing the value of pneumococcus vaccine as they had been at Camp Upton. The pandemic of influenza swept over the camp in the midst of the experiment and, because of the lowered resistance which the influenza virus induced, a certain amount of pneumonia of all types developed among the vaccinated men. Furthermore, the pneumonia, which accompanied the influenza epidemic was due in great part to Type IV pneumococcus and streptococcus, neither of which organisms had been included in the vaccine. The results obtained at Camp Wheeler, while not so successful as thoe at Camp Upton, were, nevertheless, quite encouraging. Four-fifths of the population were vaccinated, but almost as many cases of pneumonia developed among the unvaccinated one-fifth as occurred among the entire vaccinated four-fifths of camp. Reckoning from one week after vaccination, the time when the individual's immunity begins to develop, only 8 cases of Types I, II and III pneumonia occurred among the vaccinated men and all those were secondary to severe attacks of influenza. Using the same standard, 124 cases of Type IV pneumonia developed among the vaccinated troops and 103 of these were secondary to influenza. Reckoning from the day of vaccination, there were 33 cases of pneumococcus Types I, II and III pneumonia among the vaccinated four-fifths of the camp and 42 cases of pneumonia of these Types among the unvaccinated one-fifth of the camp. The death rate for 155 cases of pneumonia, including all types that developed among vaccinated troops one week or more after vaccination, was only 12.2 per cent, whereas the death rate for 327 cases of all types that occurred among unvaccinated troops was 22.3 per cent.

The author believes that even better results would have been obtained at Camp Wheeler, if a saline vaccine similar to that used at Camp Upton had been employed instead of the lipovaccine. Experiments on animals have conclusively shown that bacteria suspended in

oil do not possess as potent an antigenic capacity as when suspended in salt solution. In fact, lipovaccine has so many disadvantages that at the present time the Hygienic Laboratory of the United States Public Health Service will not issue licenses for its manufacture.

During the winter of 1918–1919 pneumococcus vaccine was used extensively in the United States Army, both in the training camps and in the American Expeditionary Forces. The following memorandum from the Surgeon General's Office in Washington is quoted from the official report of the Camp Surgeon at Camp Taylor, Kentucky (26):

January 28, 1919.

Our records show that of the 4754 men who took pneumonia vaccine only 1 case of pneumonia has developed, while in the rest of the camp there have been over 80 cases. These figures require no further elaboration and it is recommended that the inoculation be made compulsory.

TABLE 1

Results of vaccination against pneumonia

	NUMBER OF MEN		NUMBER OF PNEUMONIA CASES		DEATHS	
	Vaccinated	Not vaccinated	Vaccinated	Not vaccinated	Vaccinated	Not vaccinated
	45,849	49,463	38	83	5	11
Rate per 100,000			83.5	168	10.8	22.5

Another memorandum was submitted to the Surgeon Generals' Office in April, 1919, by Major Fred M. Meader, Medical Corps, showing the results of vaccination against pneumonia in Base Section No. 2, A. E. F. In table 1, cases were not counted unless they had developed seven days after vaccination. It will be seen from this table that both the incidence-rate and the death-rate were twice as high in the unvaccinated as in the vaccinated series.

In 1919, Major Borel (27) of the French Medical Corps made a favorable report on the use of pneumococcus vaccine among the colored troops in the French Army. It seems that these troops, coming as they did from tropical colonies, were very susceptible to pneumonia when they reached France. In one experiment three battalions were vaccinated and three others were used as a control.

The vaccine was composed of killed pneumococci suspended in normal salt solution in a concentration of 4 billion bacteria per cubic centimeter. The doses used were: (*a*) 0.5 cc. (2 billion pneumococci); (*b*) 1 cc. (4 billion pneumococci) eight days after the first injection. No reaction, either general or local, was observed among those vaccinated. The results obtained in this first experiment were very promising, although the various types of pneumococci were not contained in the vaccine.

In a second experiment conducted by Major Borel, a pneumococcus vaccine composed of several types was prepared by Professor Nicolle at the Pasteur Institute, and 300 Sengalese were vaccinated with 3 subcutaneous injections (total—28 billion pneumococci) and 300 in the same organization were reserved for controls. The result was: 1 mild case of pneumonia and no deaths among the 300 vaccinated;

TABLE 2

Incidence-rate per 1000 persons

GROUPS	TOTAL NUMBER	CASES OF PNEUMONIA	DEATHS
Vaccinated 3 times	8,306	1.0	0.5
Not vaccinated	9,388	12.0	5.5

16 severe cases of pneumonia with 4 deaths among the unvaccinated controls. The troops were under observation two months after inoculation. The author concludes that pneumococcus vaccine is of great value and that its use should be continued.

Rosenow and Sturdivant (29) vaccinated 8306 inmates of institutions with a mixed vaccine consisting of pneumococci of the four types, hemolytic streptococcus, streptococcus viridans, and staphylococcus aureus. In the same experiment 9388 persons were not vaccinated and served as a control. Table 2 shows the results obtained. It will be seen from these figures that both the incidence-rate and the death-rate were materially decreased in the vaccinated series.

Von Sholly and Park (30) vaccinated 1536 persons in the employ of the Metropolitan Life Insurance Company with a mixed vaccine directed primarily against the milder respiratory infections. A control of 3025 persons remained unvaccinated. This vaccine had

practically no effect on the incidence of influenza and colds, the rate remaining about the same in both groups. The vaccine contained pneumococci of the three fixed types, streptococci, and influenza bacilli. The interesting feature of this experiment was that only one case of pneumonia developed among the 1536 vaccinated employees, while 11 cases, or five times as many, occurred among the unvaccinated controls.

The only report on pneumococcus vaccine which has not been entirely favorable is that of McCoy, Hasseltine, Wadsworth and Kirkbride (31). These investigators studied the value of prophylactic vaccination against pneumonia among the inmates of certain New York institutions. The vaccine used was a lipovaccine containing approximately 10 billion each of pneumococcus Types I, II and III. A single dose of 1 cc. was administered subcutaneously to 17,752 patients, while 18,595 remained unvaccinated. The patients were under observation approximately two years or rather during two pneumonia seasons. Among the vaccinated half, 253 cases of pneumonia developed, while 340 cases occurred among the uninoculated. Of these cases only 122 in the vaccinated series and 186 in the control series were studied bacteriologically. An analysis of the bacteriological findings in this study is very interesting and possibly explains why more convincing results were not obtained. In the control series, only 23.6 per cent of typed cases fell into the groups of pneumococci (Types I, II and III) against which the vaccine had been directed; 76.4 per cent of the cases being caused by other organisms—pneumococcus Type IV, streptococcus, B. influenza, Friedlander's bacillus, etc. In the vaccinated series, only 18 per cent of the classified cases fell into the fixed types of pneumococcus. It should be noted further that, of the 22 cases of fixed type pneumonia that developed among the vaccinated patients, 16 were classified under pneumococcus Type III, the group which in civil life is most rarely encountered and against which, in animal experiments, it is most difficult to immunize. After making all allowances, however, it is noteworthy that, among 17,752 persons vaccinated against pneumonia and under observation for two years thereafter, there occurred only 1 case of pneumococcus Type I pneumonia, and only two cases of pneumococcus Type II pneumonia! Of course there may have been a

few more of these types among the unclassified cases. It is a well-known fact that the pneumonia which occurs in institutions for the insane, or, for that matter, in any institution, is nearly always of the bronchial type and presumably of streptococcus or pneumococcus Type IV origin. At Saranac Lake lobar pneumonia is practically never encountered in sanitariums for tuberculous patients. My criticism of this experiment, then, is that it was not a fair test for pneumococcus vaccine in that the vaccine was not directed against the type of pneumonia which was prevalent in these institutions.

Preparation of pneumococcus vaccine

The original pneumococcus vaccine used by Wright and by Lister was prepared from cultures grown on plain or glucose agar. Most of the pneumococcus vaccine manufactured in this country has been prepared from broth cultures.

Pneumococci are cultivated for eighteen to twenty-four hours on plain or glucose broth. The culture is then centrifuged, and the sediment of bacteria suspended in normal salt solution. Finally the saline solution is heated at 55°C. for one-half hour to kill the pneumococci, and the vaccine standardized by the Wright method or by means of a nephelometer. Cultures are taken to test the sterility of the vaccine and tricresol is added to a concentration of 0.3 per cent as a preservative.

Methods of administering pneumococcus vaccine

Pneumococcus vaccine is almost always administered subcutaneously. The proper method of giving the vaccine is to pinch up the skin and insert the needle well under the dermis. Intracutaneous injections excite severe local reactions. Lister experimented with intravenous injections of pneumococcus vaccine and the writer has tried it on several occasions. The intravenous method would be the method of choice were it not for the danger of exciting constitutional reactions. The immune response to intravenous vaccination is quicker and more marked than that to subcutaneous injections. The intravenous injections, however, are apt to excite the so-called non-specific protein reaction (chill, fever, leuckocytosis, etc.) similar to that following the intravenous injection of typhoid vaccine. As a

matter of fact, pneumococcus vaccine when injected intravenously is much less likely to induce a foreign protein reaction than the same dose of typhoid vaccine. The writer, however, does not recommend the intravenous method of vaccination for the reason that adequate immunity can be obtained by subcutaneous injections.

Dosage

For therapeutic purposes, pneumococcus vaccine is administered in doses varying from 10 million to 1 billion pneumococci or even more. For prophylaxis, much larger doses are used. The vaccine as prepared at the United States Army Medical School contained equal parts of pneumococcus Types I, II and III. In the United States Army, 3 to 9 billion was the dose of saline vaccine; 30 to 40 billion of the lipovaccine. In the case of saline vaccine, three injections were given at seven day intervals; the first dose, 3 billion; the second, 6 billion; and the third, 9 billion. In civil life we have used a vaccine consisting of equal parts of pneumococcus Types I, II and III, suspended in salt solution, so that 1 cc. contains a total of 9 billion killed bacteria. Three injections are given, separated by intervals of one week, as follows:

First injection	0.3 cc. (3 billion)
Second injection	0.6 cc. (6 billion)
Third injection	1.0 cc. (9 billion)

Reactions

Both the local and general reactions to pneumococcus vaccine vary greatly in different individuals. In general it may be said that the smaller the dose the milder the reaction. It is therefore desirable, if circumstances permit, to divide the total dosage (18 billion) into 4 or 5 inoculations. The writer wishes to emphasize once more, that within certain limits, the larger the total dose of vaccine administered the higher will be the immunity conferred.

In general it may be said that the local reaction to pneumococcus vaccine is similar to that following the subcutaneous injection of typhoid vaccine. Within twenty-four hours after inoculation an area of redness and induration appears at the site of injection, which is usually 2 or 3 cm. in diameter, but may be larger. The small sterile

abscesses previously described, which occasionally occur at the site of injection, are seen only in cases where large doses of vaccine have been used. These infiltrations are uncomfortable but are not serious, and disappear spontaneously. The constitutional reaction to pneumococcus vaccine is usually insignificant. In many cases it is entirely absent. In a small percentage of cases vaccination is followed by headache or backache, general malaise, chilly sensations and a rise in temperature. These symptoms, however, are generally of short duration.

Indications for pneumococcus vaccine

Pneumonia is a very prevalent disease, but hardly prevalent enough at present to justify a general and indiscriminate use of pneumococcus vaccine. There would seem to be two particular indications, however, for the use of pneumococcus vaccine; (*a*) For individuals who are very susceptible to pneumonia and suffer from repeated attacks of the disease. During the past six or seven years the writer has vaccinated a number of such persons and in no instance has the vaccine failed to give complete protection against a recurrence of the disease. (*b*) Pneumococcus vaccine is particularly valuable in the case of recruits in time of war. In previous wars intestinal infections were the chief menace to health. In the late war, pneumonia was the most serious of all the infections and caused the greatest loss of life. Pneumococcus vaccine is clearly indicated in the case of green recruits who are suddenly mustered into training camps where they are subject to contact with pneumococcus carriers, exposure to inclement weather and the lowering of resistance which comes with fatigue and overexertion. Pneumococcus vaccine could probably be used with benefit in the case of industrial workers, such as day laborers, truck drivers, chauffeurs, firemen, and policemen, who are constantly exposed to wet and cold. The practical difficulty which one encounters here is the strong objection which these people have to taking the vaccine on account of the reactions.

The advisability of vaccinating nurses, physicians and relatives who come in contact with a patient with pneumonia is a question which has not received much attention. The writer has seen a number of cases of contact infection in the same family. Pneumonia is not

looked upon as a contagious disease, but occasionally it becomes so. Once we obtain a potent vaccine which excites little or no reaction it may be worth while to vaccinate everyone who comes in contact with a pneumococcal infection.

Contra-indications

Pneumococcus vaccine should not be administered during an acute infection, or to patients with pulmonary tuberculosis. It should not be administered in large doses to patients with chronic cardiac or renal diseases, or to pregnant women. It should preferably not be administered during menstruation.

Intratracheal vaccination against pneumonia

On account of the severe reaction sometimes produced by pneumococcus vaccine when injected subcutaneously, it is clear that improvements in the method of preparation and in the method of administration will have to be forthcoming before active immunization against pneumonia will become popular in civil life. With regard to modifications in the method of administration it is quite possible that a satisfactory immunity against pneumonia could be obtained in man as in monkeys by injecting the vaccine directly into the trachea.

In a preceding section the writer has reported some experiments which throw some light on this subject. In these experiments it was found that the intratracheal injection of pneumococcus vaccine affords just as satisfactory an immunity against pneumonia as that induced by subcutaneous or intravenous injections. At the same time an attempt was made to immunize monkeys against pneumonia by spraying them with pneumococcus vaccine. Complete immunity against pneumonia was not obtained by this method, probably because the monkeys offered a great deal of resistance to the treatment and because the spray was not continued over a sufficiently long period of time. It is possible that the daily inhalation of a pneumococcus vaccine spray would prevent completely the severer forms of lobar pneumonia in man. The immunity established against pneumococcus by vaccination appears to be of rather short duration, but with an atomizer the spray could be used frequently during the winter months and permanent immunity maintained in this way.

Immunization against pneumonia with derivatives from the pneumococcus

During recent years a number of attempts have been made to isolate a pneumococcus antigen with the hope that some method might be devised for eliminating the toxic fraction of the pneumococcus and provide a more potent agent for vaccinating against pneumonia in man. Warden (32), for example, claimed that the lipoidal fractions from pneumococci contained the antigenic principle, and that with this principle he could successfully immunize animals against pneumococcus infection. Perlzweig and Steffen (33) were unable to verify the findings of Warden. They studied the antigenic value of various derivatives from the pneumococcus and found that the immunizing antigen could be isolated from the three fixed types of pneumococcus by comparatively simple chemical methods. According to these workers, this antigen in its purified form contains only a trace of nitrogen and is probably non-protein in nature. Furthermore, they found that pneumococcus antigen is resistant to prolonged autolysis, and to tryptic digestion, and can be recovered from the soluble portions of digests of either the intact bacteria or the bacterial protein. The antigen may be isolated from the 3 fixed types of pneumococcus by tryptic digestion of the pneumococci and extraction of the digest with 70 to 90 per cent alcohol. The antigen is not soluble in absolute alcohol, nor is it soluble in ether or the other lipoidal solvents. Perlzweig and Steffen concluded that pneumococcus antigen is non-lipoidal, that it probably adheres to the protein fraction in a loose chemical or physical union rather than representing a protein complex of large molecular size, as shown by its solubility in alcohol, its thermostability and its resistance to proteolytic digestion. These investigators found that purified pneumococcus antigen solutions are non-toxic for mice, and that ordinary laboratory animals can be actively immunized by them. More recently human subjects have been successfully vaccinated with this antigen. It excites little or no local reaction and the immune response is excellent.

Ferry and Fisher (34) have also studied the immunizing properties of pneumococcus antigen prepared after various methods. They found that antigen, apparently protein free, gave evidence of excellent

antigenic properties. For example, washings of pneumococci grown on agar produced an active immunity in white mice which protected them against one million fatal doses of the organism. According to these investigators phenolized cultures gave a higher antibody response than vaccines prepared in the ordinary way.

Passive immunization against pneumococcus pneumonia

In some unpublished experiments recently conducted by the writer, it was found that monkeys injected with anti-pneumococcus serum were immune to experimental pneumococcus pneumonia of homologous type when attempts were made to infect them on the day following the injection of serum. The monkeys were also immune when the serum and pneumococci were injected simultaneously. From these experiments it would appear that anti-pneumococcus serum might, like diphtheria anti-toxin, have a prophylactic as well as a therapeutic function. This is perhaps all the more feasible in view of the recent improvements that have been made in anti-pneumococcus serum (Huntoon's (35) Pneumococcus Antibody Solution; Felton's (36) Concentrated Anti-pneumococcus Serum). This method of immunization against pneumonia may prove to be of value when it is necessary to obtain immunity quickly, as for example, in patients about to be operated upon, or in patients with severe influenza or bronchitis.

SUMMARY

1. An attack of pneumococcus pneumonia confers on the patient a high immunity against the particular type of pneumococcus with which he has been infected. A small amount of cross-immunity is simultaneously established against the other types.

2. By the injection of killed or living cultures of pneumococcus, it is possible to obtain an active immunity against the homologous type of pneumococcus. This immunity is evidenced in laboratory animals by the animal's resistance to infection and by the presence of various immune bodies in the circulating blood.

3. Subcutaneous, intravenous, or intratracheal injections of pneumococcus vaccine produce in monkeys an active immunity against experimental pneumococcus pneumonia of homologous type.

4. Pneumococcus vaccine when given in adequate doses confers immunity against pneumococcus pneumonia in man. The evidence in favor of such immunity rests on statistical studies and on the demonstration of immune bodies in the blood of those vaccinated.

5. The chief indications for the use of pneumococcus vaccine at the present time are: *First*, to prevent recurrence in individuals who are highly susceptible to repeated attacks of lobar pneumonia. *Second*, for the prevention of outbreaks of pneumonia among fresh recruits in training camps.

6. There is reason to believe that the recent isolation of certain derivatives of the pneumococcus which contain the antigenic principle will eliminate the undesirable features of vaccination against pneumonia and thereby render the procedure more practical in civil life.

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VOLUME V

1926

MEDICINE

Analytical Reviews

of

General Medicine
Neurology and Pediatrics

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THE WILLIAMS & WILKINS COMPANY
BALTIMORE, MD.
1926

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THE PARATHYROID GLANDS[1]

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GROSS ANATOMY OF THE PARATHYROID GLANDS

The parathyroid glands in the human subject are bean-shaped structures 3 to 15 mm. long, and 2 to 3 mm. broad and thick. They are yellowish-brown to brown-red in color. As a general rule there are four distinct glands, situated in such relation to the thyroid lobes that it has been customary to distinguish between a superior and an inferior pair of glandules. The superior pair are the more constant in position. They occur on the medial aspect of the dorsal surface of each lateral lobe of the thyroid gland at about the junction of its upper and middle thirds. The inferior pair occur also as a rule on the dorsal surface of each lateral lobe of the thyroid gland but further caudad. Accessory parathyroids are sometimes present and these may be situated in the thorax nearer to or embedded in the thymus gland (Cowdry).

Parathyroid glands have been recognized in all classes of the vertebrates with the exception of the fishes (Cowdry).

The anatomical arrangement of these glands in certain of the mammals is a matter of considerable interest and affords an explanation for some of the contradictory results reported by some of the earlier workers in this field. In the dog and cat for example, the four glandules are so closely associated with the lobes of the thyroid that in the operation for thyroidectomy, they are completely removed whereas in the rabbit, removal of the thyroid gland results in only one pair of parathyroid glandules being taken away. The superior pair of glandules are frequently described as "external" and the inferior pair as "internal" in certain of the mammals, thus indicating that one pair of glandules are more or less removed from the thyroid lobes and that

[1] Lecture delivered before the Harvey Society in New York City on January 2, 1926.

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one pair are imbedded in the lobular tissue of the thyroid. Accessory parathyroids are also very common in certain of the lower mammals.

MINUTE ANATOMY AND EMBRYOLOGICAL DEVELOPMENT OF THE PARATHYROID GLANDS

The parathyroid gland possesses a capsule of fibrous connective tissue. The glandular cells within the capsule are disposed in columns or clumps, which are separated to a greater or lesser extent by bands of connective tissue which are continuous with the capsule. Two types of glandular cells are usually recognized. The parenchyma consists in the main of the so-called chief cells of large polygonal type which are characterized by their clear cytoplasm and faintly staining nuclei. The second type known as the oxyphil cells, stain strongly with eosin and the nucleus with hematoxylin.

The parathyroid glands develop as thickenings of the endoderm of the third and fourth branchial clefts. The superior pair of glandules are related to the fourth branchial clefts developmentally while the inferior pair are similarly related to the third branchial clefts.

DISCOVERY OF THE PARATHYROID GLANDS

The superior pair of parathyroid glands was first described by Sandström in 1880. He found these bodies constant in fifty autopsies in man and he also studied and accurately described their position and structure in the dog, cat, rabbit, ox and horse. Sandström's important contribution was lost sight of and it was not until eleven years later when Gley rediscovered the superior pair of parathyroid glands that due attention was called to these small structures.

Kohn (1895) discovered the inferior or internal pair of parathyroid glands. This author recognized that every animal is provided with four parathyroid glands occurring in two pairs. An internal pair present in the substance of the thyroid and an external pair, situated at some distance from the thyroid in the herbivora, but in the carnivora lying on the surface of that gland and usually within its capsule. Kohn insisted that the parathyroid glands are organs anatomically separate and functionally distinct from the thyroid. In this he differed from Gley who held at first that the parathyroid glandules represented embryonic thyroid tissue.

PHYSIOLOGY OF THE PARATHYROID GLANDS

Historical

The earliest recorded experiments on animals bearing upon the physiology of the parathyroid glands are those of Raynard (1835). Raynard removed the thyroid gland from a number of dogs and found that death frequently resulted in the course of a few days.

Schiff (1859) removed the thyroid gland from a number of animals of different species—rabbits, rats, dogs, guinea pigs and fowls and found that while some survived, others died within a few days. In 1884 he published the results of a more extensive investigation. He states in this communication that no symptoms followed removal of the thyroid gland in the case of rats and rabbits whereas in the dog and cat, the result was almost invariably fatal.

When Gley (1891) later discovered the external pair of parathyroid glands, it became apparent to him that thyroidectomy as previously practiced meant in the case of the dog and cat parathyroidectomy as well, and the divergent results obtained by Schiff following thyroidectomy in different species of animals came to have a rational explanation. Gley carried out a series of experiments the results of which demonstrated that the acute symptoms manifested by certain animals following thyroidectomy are due to removal of the parathyroid glands along with the thyroid. Vassale and Generali (1900) removed the four parathyroids from ten cats and nine dogs, leaving the thyroid gland almost intact. Of the cats so treated nine died within ten days, the majority dying about the fifth day. All of these presented a typical syndrome, viz., fibrillary contractions and muscular spasms, psychical depression, rigid and uncertain gait, anorexia, tachycardia, rapid emaciation, fall in body temperature and death. Of the nine dogs in this series all died within eight days, most perishing on the third or fourth day. All were in good condition on the day following the operation. Symptoms began to appear on the second or third day and death resulted shortly thereafter. The symptoms recorded were tremors, psychical depression, paresis of the muscles of mastication, trismus, rigidity of the hind limbs, uncertain and spastic gait, muscular weakness and convulsions. There was loss of appetite and vomiting, palpitation and dyspnea; the urine was scanty and some-

times showed traces of albumin. At post-mortem examination, slight congestion of liver and kidneys was noted but nothing specific to point to the cause of death was found. They adopted the view that the parathyroid glands exercise normally an anti-toxic function and that their removal causes death by poisoning. Since the work of Vassale and Generali, numerous investigators have demonstrated the vital nature of the parathyroid glands. The recent work of Nicholas and Swingle (1925) on cats would seem to establish conclusively that complete removal of the parathyroid glands is a direct cause of a specific syndrome of symptoms and death.

Apart from this one fully demonstrated fact that the parathyroid glands are vital structures, the physiology of these glands has been quite obscure. There have been two main theories of parathyroid function and each of these has had its adherents. These theories will now be discussed briefly.

THE TOXIN THEORY

As has been already stated Vassale and Generali (1900) attempted to explain the cause of parathyroid tetany by assigning an anti-toxic function to the parathyroid glands. The thyroid gland, they held, stimulated metabolism. The more intense the metabolism, the more toxic substance would be produced. In this manner they fitted a number of outstanding experimental observations to their theory. Among these latter observations were the following: Parathyroidectomy is less serious and runs a milder course in old than in young dogs. It is extremely severe in dogs that eat heavily, particularly of meat, and in fasting animals the symptoms are much less severe.

Many attempts were later made to demonstrate the hypothetical tetany toxin in the blood of parathyroidectomized animals. None of these attempts have been attended with clear cut results. Biedl and others have reported that copious bleeding and the transfusion of normal blood relieves the symptoms of tetany in parathyroidectomized animals. It is argued from these results that a toxin present in the blood is responsible for the symptoms and by the process of blood-letting the organism may be temporarily freed from the effects of the poison.

MacCallum (1909) observed that the withdrawal of approximately

one-third of the blood from an animal in the acute stage of parathyroid tetany and the replacement of the same with normal saline solution caused the symptoms to be promptly relieved. This was confirmed by Berkeley and Beebe (1909). These latter investigators believed that parathyroid tetany is due to a toxic product of metabolism derived from the decomposition of albumin. The fact that the symptoms are considerably aggravated in meat fed dogs confirmed them in this belief. Joseph and Meltzer (1911) showed that the injection of sodium chloride solution without previous bleeding will allay the neuromuscular symptoms. Koch (1912) and 1913) described the presence of methyl-guanidine and other bases in the urine following parathyroidectomy.

Paton and his collaborators later claimed to establish the following points:

1. That the symptoms produced by guanidine and methyl-guanidine are identical with those seen in operative and idiopathic tetany.

2. That very small doses of these substances markedly aggravate the symptoms of tetania parathyreopriva.

3. That their amount in the blood and urine is increased in experimental tetany and in the urine in idiopathic tetany.

Findlay and Sharpe (1920), and Nattrass and Sharpe (1921) have observed an increased amount of guanidine, apparently as di-methyl-guanidine in the urine in cases of tetany in adults. Paton (1924) states that the mass of evidence indicates that complete removal of the parathyroid tissue leads to a fatal toxemia; he further hypothecates that the parathyroids through their internal secretion control the tone of muscles by regulating metabolism, i.e., the production and destruction of guanidine in the body. The views of the Glasgow school have received support from the work of Frank, Stern and Notthmann, Gyorgy and Vollmer, Palladin and Griliche and others.

It must be admitted that a very strong case has been made out for the guanidine intoxication theory of parathyroid tetany. The evidence, however, consists so largely of the circumstantial type that it fails to carry with it the weight of final conviction.

THE CALCIUM THEORY

J. Loeb (1901) showed that the injection of any salt that precipitates calcium causes muscular twitchings.

MacCallum and Voegtlin (1909) demonstrated in the condition of parathyroid tetany, a lowered calcium content of the tissues and body fluids, especially of the blood, the calcium content of which might be reduced to as much as 50 per cent of its normal value. They also showed that calcium salts injected intravenously caused the convulsions of tetany to cease and the animal to be restored to a normal condition. Oral and subcutaneous administration of calcium salts had a similar effect although the beneficial action developed much more slowly. They concluded that the function of the parathyroid glands is to regulate the calcium exchange in the body and considered that all the symptoms following parathyroidectomy are due to calcium deficiency.

MacCallum (1912) showed that the galvanic hyperexcitability of the nerves which is a characteristic feature of tetany is due to some change induced in the blood by parathyroidectomy. By causing the blood from a tetany dog to flow through the leg vessels of a normal dog he demonstrated that an excitability identical with that found during tetany appears in the nerves of the normal leg.

Later MacCallum, Lambert and Vogel (1914) showed quite conclusively by means of dialysis experiments that the change in electrical excitability of nerves in the tetany state is due to diminished calcium in the blood.

Luckhardt and Goldberg (1923) showed that parathyroidectomized dogs can be kept free from symptoms and in good health even on a meat diet when calcium therapy is pushed far enough.

Salveson (1923) obtained somewhat similar results and related the whole symptomatology of parathyroid deficiency to a lowered calcium content of the blood. The calcium content of the blood he held is normally controlled by the parathyroid glands.

Collip (1925) reported results in accord with these latter authors.

Clinically the immediate relief of symptoms following the intravenous administration of calcium salts in low blood calcium tetany, has been repeatedly confirmed.

MISCELLANEOUS OBSERVATIONS BEARING UPON THE PHYSIOLOGY OF THE PARATHYROID GLANDS

Acid-base equilibrium

Wilson and his co-workers (1915) considered that deranged metabolism whereby the normal equilibrium between acids and bases in the blood is disturbed, is the cause of the symptoms of parathyroid tetany. They made a careful study of the ammonia excretion in parathyroidectomized dogs and found that the ammonia output is diminished at first but later increases when the convulsions start. Since the excretion of ammonia is dependent on the amount of acid which has to be excreted by the kidney, these authors concluded that a condition of alkalosis develops following the removal of the parathyroid glands. This condition they held is relieved by the development of a state of acidosis caused by acid formation during the convulsions. In confirmation of this, actual changes in blood reaction were demonstrated in the pretetany and tetany states.

Collip and Backus, and Grant and Goldman (1920) demonstrated that by voluntary hyperpnea, a state of tetany is produced. Forced ventilation of the lungs produces a state of alkalosis and in this condition, however produced, symptoms of tetany may be manifested. As the blood calcium may be perfectly normal in the tetany of alkalosis, it may be assumed that the symptoms result from a decrease in calcium ions which is an inevitable result of an increased pH of the blood.

Tetanic seizures have been produced by the disturbance in the kation equilibrium of blood or spinal fluid as by the injection of sodium chloride (Collip (1920) and Greenwald (1922)). It is doubtful if this type of experimental tetany bears any relationship to parathyroid tetany. The tetany of alkalosis does however bear a very direct relationship to parathyroid tetany. The administration of acids has been shown to have a definite beneficial effect in parathyroid tetany. This action of acids may be due to an increase in the ionization of the blood calcium resultant on their use. It has been our experience that, in about 50 per cent of untreated parathyroidectomized dogs, the outstanding prodromal sign of an approaching tetanic seizure is violent hyperpnea. The animals in this group and manifesting this

sign, are subject to tetanic seizures of the most violent sort and death results as a rule earlier than in the other group. Cameron and Carmichael (1925) report somewhat similar results. From these findings, it may be concluded that alkalosis may play a definite rôle in parathyroid tetany but it is not a necessary concomitant. When it is present the symptoms are exaggerated and early death is more likely to occur.

The intestinal factor

Much evidence has been brought forward to show that both diet and the type of flora of the alimentary canal play a very important rôle in relation to the development of tetany in parathyroidectomized animals. Thus it has long been known that a meat diet precipitates the onset of symptoms and increases their severity while a milk diet decreases the severity or prevents the development of symptoms. Dragstedt and Peacock (1923) obtained results which indicated to them that the symptoms of parathyroid tetany are primarily the result of the absorption of the products of bacterial decomposition of meat in the intestine due to the development of a special proteolytic flora as a result of a meat diet. Their experiments also indicate, as have those of others, that tetany is much less marked when animals are given a milk diet. They also found that lactose and dextrin seem to have a special action in delaying the onset of symptoms. This action they have ascribed to the establishment by these food stuffs, of an aciduric flora in the intestine which prevents the bacterial decomposition of proteins into toxic split products.

Luckhardt and Compere (1924) have pointed out that changes in the permeability of the mucosa of the gut may have an important bearing in relation to parathyroid tetany.

That the liver plays an essential rôle in the development of the so-called toxemia of parathyroidectomized dogs has been argued by Blumenstock and Ickstadt (1924). These authors found that parathyroidectomy in Eck fistula dogs resulted in a delay in the appearance and a diminution in the severity of the characteristic symptoms. When symptoms did appear they were abolished with the greatest ease by the administration of calcium salts per os. They suggest that substances absorbed from the gut are not the direct toxic agents as such, but they may cause tetany after being modified by some tissues, most probably the liver.

The beneficial effect of intravenous saline

The fact that bleeding[2] and the transfusion of normal blood is highly beneficial in parathyroid tetany coupled with the observation that intravenous injections of normal saline have a similar good effect, have done much in the past to discredit the calcium deficiency theory of tetany. Luckhardt and Rosenbloom (1921) made the case against the calcium theory much stronger when they showed that timely intravenous injection of calcium free Ringer solution prevents or controls tetany in parathyroidectomized dogs over a considerable period of time. With such treatment the calcium content of the blood must be still further diminished and yet the effect is beneficial.

The blood chemistry in parathyroid tetany

The most constant finding in the blood condition in parathyroid tetany is a greatly diminished calcium content.

Nearly all investigators agree that in the course of tetany there is a retention of phosphorus in the blood (Greenwald, Elias and Weiss, Elias and Spiegel). Greenwald has emphasized that the decrease in phosphorus excretion and the increase in blood phosphorus are out of proportion one to the other. He finds a considerable decrease in the excretion of phosphorus in parathyroidectomized dogs but only a slight increase in the inorganic phosphorus of the blood, a fact pointing to retention of phosphorus elsewhere than in the blood.

Underhill and Blaterwick (1914) observed hypoglycemia in dogs following parathyroidectomy. Salveson (1923) failed to confirm this. Our own results are in accord with Salveson in this matter.

Togawa (1920) found a condition of acidosis in dogs suffering from parathyroid tetany and also noted that the non-protein nitrogen of the serum is usually increased. Little importance can be attached to these latter results since the average control non-protein nitrogen value reported is 77 mgm. per 100 cc. of serum.

[2] Swingle and Wenner (Amer. J. Physiol., lxxv, 372) have just reported that the withdrawal of considerable quantities of blood from dogs suffering from tetania parathyropriva promptly relieves the symptoms and induces a marked rise in the level of serum calcium. They state that the abatement of symptoms coincides with the rise in calcium and that within 10 or 12 hours after bleeding the calcium again decreases in the blood and tetany again appears.

Paton and his co-workers (1924) find an increase in methyl-guanidine in the blood.

Greenwald (1924) was unable to demonstrate any toxin in the blood of parathyroidectomized dogs and is of the opinion that no toxin exists.

Kramer, Tisdall and Howland (1921) found in infantile tetany that the inorganic phosphorus shows marked variation. They found that the concentration of sodium and magnesium is essentially normal while potassium is slightly increased. Calcium is lowered and is the one inorganic constituent of tetany blood, the concentration of which is markedly changed.

EXPERIMENTAL TETANY

Tetany has been produced in animals by various experimental measures. As the study of experimental tetany induced by means other than parathyroidectomy may throw some light on parathyroid tetany itself a number of the experimental tetanies will be briefly referred to:

1. Any salt which will precipitate calcium in the tissues and body fluids causes muscular twitchings (Loeb (1901)).

2. Sodium chloride given in massive doses by intravenous injection will produce tetanic seizures (Greenwald).

3. The intravenous injection of sodium bicarbonate has been observed on occasion to cause tetanic convulsions in both man and experimental animals (Tileston, Harrop, and others).

4. Morris (1922) has demonstrated that all conditions of anoxemia markedly increase the electrical excitability of nerve endings.

5. Campbell (1925) has very recently suggested that tetany and convulsions are caused by oxygen deficiency in the cell (brain, spinal cord) and that the purpose of tetany and convulsions is to counteract this defect.

6. Guanidine and methyl-guanidine produce a state of tetany somewhat similar to that seen in parathyroid deficiency. The action of these bases is cumulative (Paton).

7. Forced ventilation of the lungs is productive of mild tetany (Collip and Backus (1920); Grant and Goldman (1920)).

8. Gastric tetany has been produced in dogs by McCann (1918).

9. Binger (1917) showed that the injection of phosphates in sufficient amount would cause a decrease in the calcium content of the blood and produce tetany provided neutral or alkaline solutions were used. Acid sodium phosphate caused a decrease in the blood calcium but did not produce tetany.

10. Cameron and Carmichael observed tetany in a certain percentage of rats which had been fed desiccated thyroid gland.

11. Tetany can be produced in rats by dietary measures alone (low calcium and high phosphorus).

12. Tetany is manifested in rachitic rats.

13. There are at least three factors which influence the production of experimental tetany, viz., tissue anoxemia, disturbance in ionic equilibrium and pH.

TETANY AS A CLINICAL ENTITY

Tetany is a clinical syndrome characterized by a peculiar hyperexcitability of the nervous system (motor, sensory and autonomic) and in manifest cases, also, by spontaneous attacks of peculiar tonic spasms, involving certain groups of muscles, or even the whole body musculature.

The syndrome can be easily and surely recognized, in all cases by demonstrating the existence of its most constant and characteristic mark, namely, an increased response to galvanization of the motor nerves (Erb's sign) (Barker).

Certain signs are usually present. Most important of these are the Trousseau and Chvostek phenomena. Pressure on the nerves in the bicipital sulcus gives rise to a peculiar obstetrical attitude of the hand and forearm (Trousseau). Tapping the facial nerve causes contractions of the muscles on the corresponding side of the face (Chvostek). Carpo-pedal spasm is usually present in manifest tetany.

TYPES OF TETANY

Infantile tetany

This condition is most commonly seen in the so-called tetany months of March and April.

Post-operative tetany or *tetania strumipriva* occurs as a result of

removal of or damage to (cutting off of blood supply) the parathyroid glands.

Both infantile tetany and post-operative tetany are probably conditions similar to parathyroid tetany in animals. Both show a lowered calcium content of the blood and both types yield to calcium therapy.

Gastric tetany

This condition was first described by Kussmaul. The blood calcium is normal in this type of tetany and the underlying causative factor would appear to be an alkalosis set up as a result of the withdrawal from the body of excessive amounts of acid.

Tetany due to hyperpnea

Tetany due to excessive pulmonary ventilation has been reported (Barker and Sprunt (1925)).

Other forms of tetany in the adult human subject are tetania gravidarum and idiopathic tetany.

PARATHYROID GRAFTS

There are a number of cases of successful parathyroid grafts recorded in the literature. Schiff (1884) succeeded in keeping a thyroidless dog alive by the abdominal implantation of fresh thyroid glands. Eiselsberg (1892) showed more positively that the thyroid gland when implanted into the abdominal fascia or in the peritoneum of cats, healed in and prevented the occurrence of tetany. When the healed-in-tissue was removed tetany followed and the animal died.

Halsted (1909) has described the successful transplantation in dogs of both their own parathyroid glands and those of other dogs.

Successful transplantations of human parathyroid glands have been reported by a number of workers.

Borcher (1919) states that a single parathyroid gland is adequate to obviate symptoms in the human subject.

PARATHYROID EXTRACTS

Moussu (1898) claimed to have arrested post-operative tetany in dogs by subcutaneous and intravenous injections of extracts prepared

from the parathyroid glands of the horse. He used sterile water and glycerin to prepare his extracts.

Vassale (1905) introduced an extract of parathyroid glands which he called parathyroidin. He asserted that it had a favorable effect upon tetany attacks in human beings. His method of preparing the extract was not published.

Vincent and co-workers (1905) were unable to prevent the onset of symptoms in thyroparathyroidectomized animals either by the use of thyroid or parathyroid gland extracts.

Berkeley and Beebe (1909) prepared an extract from beef parathyroid glands by the use of acetic acid, from which they separated a nucleoprotein. This parathyroid nucleoprotein they found to have curative properties when administered to parathyroidectomized dogs.

Berman (1924) published a short note in which he claimed to have isolated by acid alcoholic extraction of parathyroid glands a crystalline substance which raised the blood calcium when injected into rabbits.

MacCallum (1924) in a paper dealing with the pathogenesis of tetany refers to the use of parathyroid gland preparations as follows:

> Extracts of parathyroid glands have naturally been tried, both experimentally and clinically, throughout many years and some of them seem to have some effect in restoring calcium balance and the normal excitability of nerves, but at best it is a slight and questionable effect and less satisfactory in experimental animals than in the tetany of adults, from which it may probably be assumed that the psychic effect of any treatment plays a part there.

Hanson (1923–1924) has published a series of papers in which he describes the preparation and use in both experimental animals and man of a weak hydrochloric acid extract of ox-parathyroid glands. He prepared the extract by first extracting the glands with boiling, dilute hydrochloric acid. Many of Hanson's results are highly suggestive of the presence of an active principle in his extract. No absolute scientific proof, however, is given of the presence of the parathyroid hormone in these extracts.

Collip (1924) brought forward final proof that extracts prepared by weak acid hydrolysis of the fresh or acetone preserved parathyroid glands of the ox contain the active principle of these glands. This

proof consisted in the demonstration that such extracts consistently and uniformly prevent or relieve tetany in thyroparathyroidectomized dogs. This result was obtained in the case of young dogs kept on a lean meat diet, and treated with alkalinized extracts. It was also shown that the probable manner of action of the hormone contained in these extracts is through a direct effect on calcium metabolism. The blood serum calcium of parathyroidectomized dogs is found to be elevated following the use of the extract. The injection of the extract into normal animals causes the blood serum calcium value to be elevated and a method of assay was later (Collip and Clark (1925)) developed based upon this finding.

These results have been confirmed by a number of workers, Macleod and Taylor, Cameron and Moorehouse, Greenwald,[3] Fisher and Larson, and Hjort.

PREPARATION OF THE HORMONE

Fresh glands are ground in a meat chopper, placed in large Pyrex tubes (5 + 45 cm.) and covered with an equal volume of 5 per cent hydrochloric acid. Three per cent hydrochloric acid is used if the glands have been preserved in dry acetone. The tubes and their contents are then placed in a boiling water bath for from thirty minutes to one hour. During the digestion the mass is stirred and broken up with a glass rod until most of the material is in solution, and that which is not, quite finely divided. When this treatment is finished, the extract is diluted with four parts of hot water and set aside to cool. The fat, which separates and rises to the top of the container, congeals upon cooling and is removed mechanically. The liquid is thereupon made alkaline (pH 8.0 to 9.0) with sodium hydroxide which dissolves practically all the suspended material. Hydrochloric acid is then added with constant stirring until considerable precipitation occurs which, at the same time, permits rapid filtration. The object to be attained here is to have the liquid at a hydrogen ion concentration such that as much as possible of the active material is in solution, but at the same time rapid filtration is possible. Usually the hydrogen ion concentration is in the neighborhood of pH 5.5 to 5.6, but the

[3] Personal communication.

proper condition can best be determined by trial. After the liquid is filtered the precipitate remaining on the filter is dissolved in weak alkali and again treated with hydrochloric acid as before. This process is repeated as long as any active material remains in the filtrates. This point is determined by noting whether upon making the filtrate acid to congo red and saturating it with sodium chloride an appreciable amount of precipitate is formed. The active substance is separated from the filtrates by salting-out with sodium chloride, the first filtrate being worked up separately from the subsequent ones. The salting-out is done by making the solutions acid to congo red and saturating them with sodium chloride. The substance generally flocks out and rises to the top of the liquid. The precipitate is transferred to a filter, separated from its mother liquor, dissolved in weak sodium hydroxide, centrifuged, and the liquid adjusted to pH 4.8. The isoelectric precipitate which thus separates is either filtered or centrifuged off, dissolved by the addition of hydrochloric acid, and the process repeated until the mother liquors are quite clear and devoid of color. The substance is dissolved in hydrochloric acid at pH of about 3, Berkefelded, standardized, and is then ready for use.

A very satisfactory preparation for laboratory use can be made from the original residue. Even after several extractions of this residue as above described, considerable of the active principle remains behind. This was at first overlooked since the washings which were found to be inert were made at a pH near to the isoelectric point. The residue has been worked up as follows. It is taken into solution by the addition of acid, sodium chloride is then added until flocculation occurs. The precipitate is removed and is then submitted to a process similar to that described.

THE CHEMISTRY OF THE ACTIVE EXTRACT

The chemical properties of the most highly purified active extract of the parathyroid glands of oxen which has been obtained are such as to indicate that it consists essentially of a protein-like substance. It gives the common protein reactions, namely, the xanthoproteic, Millon's, biuret, ninhydrin and Hopkins-Cole reactions. The Molisch carbohydrate reaction and the orcinol-hydrochloric test for pentose are not given. Sulphur and iron are present (Collip and Clark).

There is the possibility which cannot be overlooked that the active principle may be some fairly simple compound which associates itself with the particular protein-like fraction which has been dealt with. The fact, however, that proteolytic enzymes completely inactivate the most potent preparations argues against this possibility. It has also been found that no appreciable change in chemical composition or in physiological activity occurs when repeated isoelectric precipitation of already purified material is carried out. Thus in one experiment, material was purified by two precipitations with sodium chloride and ten isoelectric fractionations. The solid was washed with water at pH 4.8 and recovered by centrifuging. It was then suspended in absolute alcohol of such amount that the resulting concentration of the latter substance was 95 per cent. An equal volume of dry ether was added to the alcoholic suspension and the precipitate was recovered by centrifuging. The solid as obtained was washed with dry ether and finally the ether was removed and the substance was thoroughly air dried. On analysis, the air dried powder contained 14.5 per cent of nitrogen. Similar material which had been dried in vacuo over sulphuric acid and potassium hydroxide contained 15.5 per cent of nitrogen thus indicating that the purified powder is hydroscopic. The purified solid prepared as outlined above, was taken into solution in weak acid and precipitated isoelectrically. This process was thrice repeated. The precipitate was then treated with alcohol and ether as in the first instance. The air dried resultant solid contained 14.6 per cent of nitrogen and when submitted to the physiological test for potency showed no appreciable change (Collip and Clark).

The purified material in the form of the desiccated solid or in weak acid solution (sterile) has been found to be stable. The purified solid is soluble in absolute alcohol to the extent of 0.1 per cent. It is insoluble in acetone, ether and pyridine. Its physiological activity is completely destroyed by boiling for one hour with 10 per cent hydrochloric acid or 5 per cent sodium hydroxide. It is rendered inert by the action of both pepsin and trypsin. In order to be assured that the action of these four reagents was to effect complete destruction of the physiologically active material, a number of dogs were injected at frequent intervals over a period of many days with massive doses of this highly potent extract. This extract, divided into four lots, had

been treated respectively with 10 per cent hydrochloric acid, 5 per cent sodium hydroxide, pepsin and trypsin. Frequent blood serum calcium determinations were made and in no instance was any evidence obtained of the presence of the active principle in any of the treated extracts.

The active principle does not dialyze through collodion membranes to any appreciable extent.

It is removed from solution by Norit and by the Folin-Wu tungstic acid reagent.

Chemically the purified solid resembles insulin somewhat. Attempts to further purify it by the method recently employed so successfully in the case of insulin by Abel and his co-workers have not met with success in our hands. That it will ultimately be purified to a much greater extent would seem, however, an inevitable conclusion.

THE PHYSIOLOGICAL PROPERTIES OF THE ACTIVE PRINCIPLE

The outstanding physiological property of the active principle of the parathyroid glands is its specific effect on the concentration of calcium in the blood. There may be other direct effects of the active principle but this is the only one which has been absolutely proven. It is possible that all other effects resultant on the experimental production of hyperparathyroidism by means of the hormone, are purely secondary to the changes directly induced in calcium metabolism. Changes which occur in the last few hours of life in a dog for example which is in an essentially moribund condition as a result of parathyroid hormone overdosage, should not be interpreted as direct or immediate effects of the hormone. They are rather preterminal phenomena which have little practical bearing on the purely physiological effect of the hormone itself. The physiological action of the parathyroid hormone is normally to regulate calcium metabolism and to maintain a definite level of calcium in the circulating blood.

THE EFFECT OF THE INJECTION OF THE PARATHYROID HORMONE

Normal animals

There is an enormous variation in the response of different normal animals to injections of the parathyroid hormone. Single small

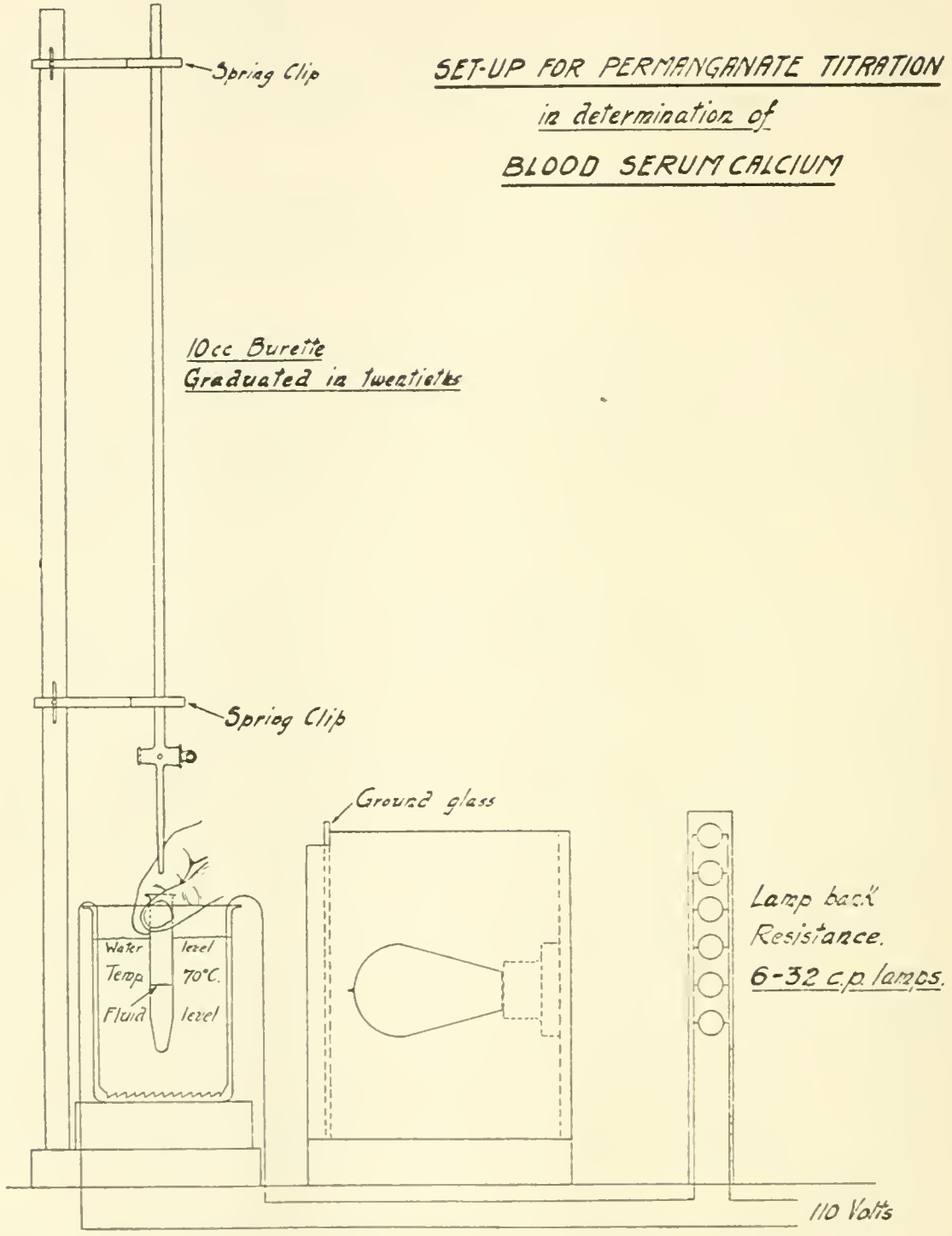

FIG. 1. SET-UP AS USED FOR PERMANGANATE TITRATION IN ESTIMATION OF BLOOD SERUM CALCIUM

doses have produced no objective effects in those animals so treated. The response to repeated injections at short intervals of time is much different. Some animals exhibit a typical train of symptoms which end fatally if the injections are continued past a certain point while others are relatively immune. Considerable work still remains to be done in this field. It would appear that the dog is of all animals the most susceptible to "poisoning" by overdosage. The cat responds in a somewhat similar manner but this animal is relatively slightly more resistant to the active extract than are dogs. Rabbits are practically

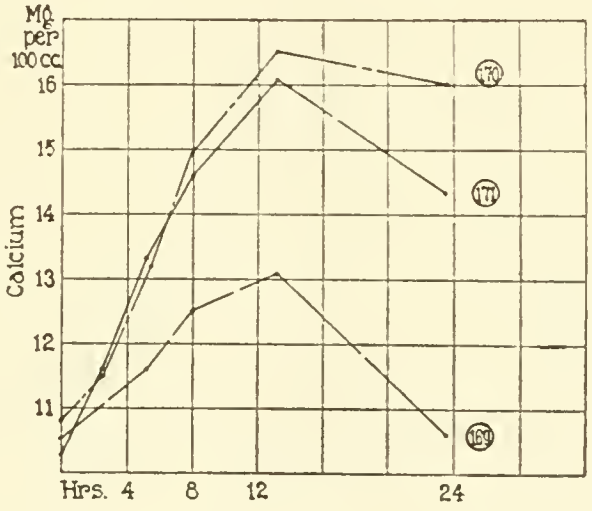

CHART 1. Showing that the response to single subcutaneous injections of parathyroid hormone is of a quantitative nature.

Dog 169—22 kilos—received 50 units.

Dog 171—20 kilos—received 75 units.

Dog 170—19 kilos—received 100 units.

immune to repeated injections of the hormone. Hens are in the same category.

The dog is the most suitable animal upon which to study the changes produced in blood chemistry following injections of the hormone. This is so for two reasons, viz., the nature of the response of this animal and the ease with which frequent blood examinations can be made.

It has been found that the main effect of single injections of active extracts of the parathyroid glands into normal dogs is an increase in the blood serum calcium value. For some hours following an injection the blood serum calcium gradually increases until a peak point is reached, a return to normal is then gradually accomplished. The

slope of the descending curve is somewhat the same as that of the ascending one. The peak point is reached in twelve to twenty-four hours after the injection but it is usually attained in from fifteen to eighteen hours.

It has been shown that the increase in blood serum calcium attained in fifteen hours is almost directly proportional to the dose administered. Thus, by using a large number of dogs and noting the blood serum calcium increases over the fifteen hours following the injections any given batch of extract can be physiologically standardized. Considerable variation is of course found in the response of different

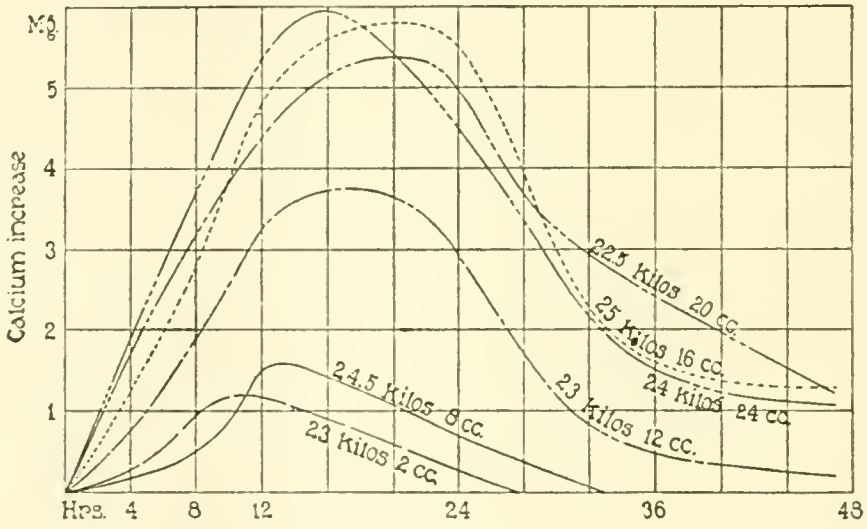

CHART 2. Constructed curves illustrating that the blood serum calcium response to a single injection is directly related to the size of the dose administered.

dogs but by using a large number of animals in a potency test these variations are compensated for to a fair degree. No absolute relationship between the weight of an animal and the blood serum calcium mobilizing effect manifested has been found. For this reason, in potency testing, animals approximating to twenty kilos in weight are used. Food is also withheld for twenty-four hours previous to the test.

Young animals seem to respond better than old animals. Probably the ideal for potency testing will be young animals of the same litter. A unit of potency has been defined as one one-hundredth of that amount of extract which produces on an average a 5-mgm. rise in blood serum calcium in 20-kilo dogs, over fifteen hours.

No marked and constant variations have so far been noted in the blood constituents of normal dogs other than calcium following single moderate doses of the hormone.

Single injections of the parathyroid hormone have not as a rule any ill effect upon dogs unless the dosage is excessive. If the calcium rises above 15 mgm. per 100 cc. of serum, vomiting may occur, but this in itself is not serious. If massive doses are administered the blood serum calcium may rise to 20 mgm. per 100 cc. or even higher

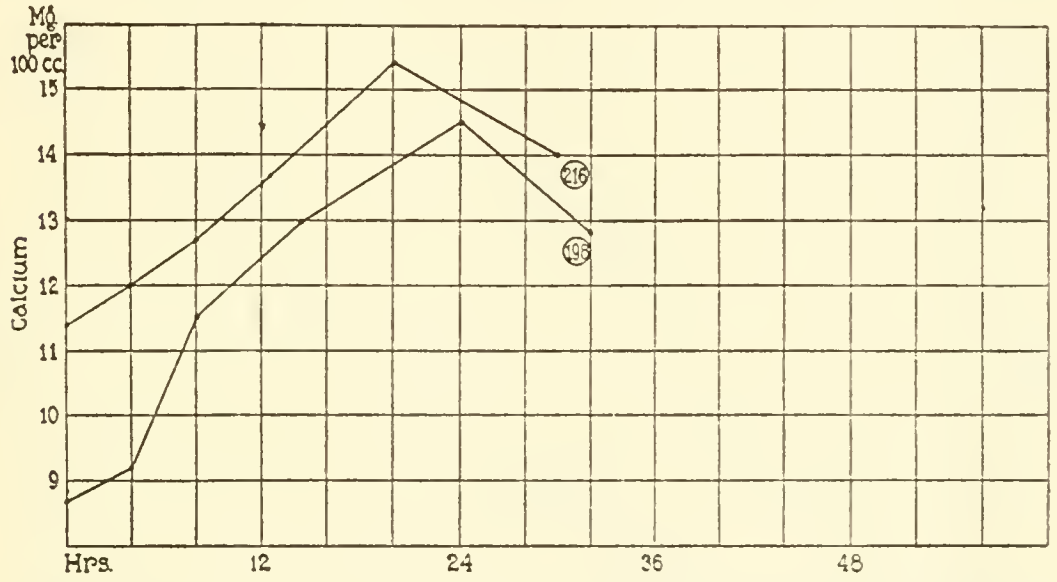

CHART 3. Showing that the response of parathyroidectomized dogs to injections of the hormone is of the same order as the response of normal dogs.

Dog 198—19 kilos—thyroparathyroidectomized.

Dog 216–18.5 kilos—normal animal.

Both animals treated exactly alike, each receiving three injections of the hormone at four-hour intervals.

and the animal may die on the second or third day. If the condition of profound hypercalcemia is not maintained for an undue length of time, uneventful recovery may occur.

THE EFFECT OF REPEATED INJECTIONS AT SHORT INTERVALS

Normal dogs injected at four-hour intervals with 25 units of parathyroid hormone manifest a typical train of symptoms, run a characteristic blood calcium curve and die within forty-eight to seventy-two hours as a rule. Certain blood constituents other than calcium

also run a characteristic curve. The physical properties of the blood are also altered. Somewhat similar results are obtained when larger or smaller dosage is administered and when the time interval between injections is varied to a considerable degree. The symptoms manifested and the order of their occurrence are somewhat as follows: Some hours after the injections have been begun the animal has attacks of vomiting followed by diarrhea; a certain uneasiness of manner may be manifested at this time but otherwise the animal is quite normal in its behavior. During this period (approximately twenty-four

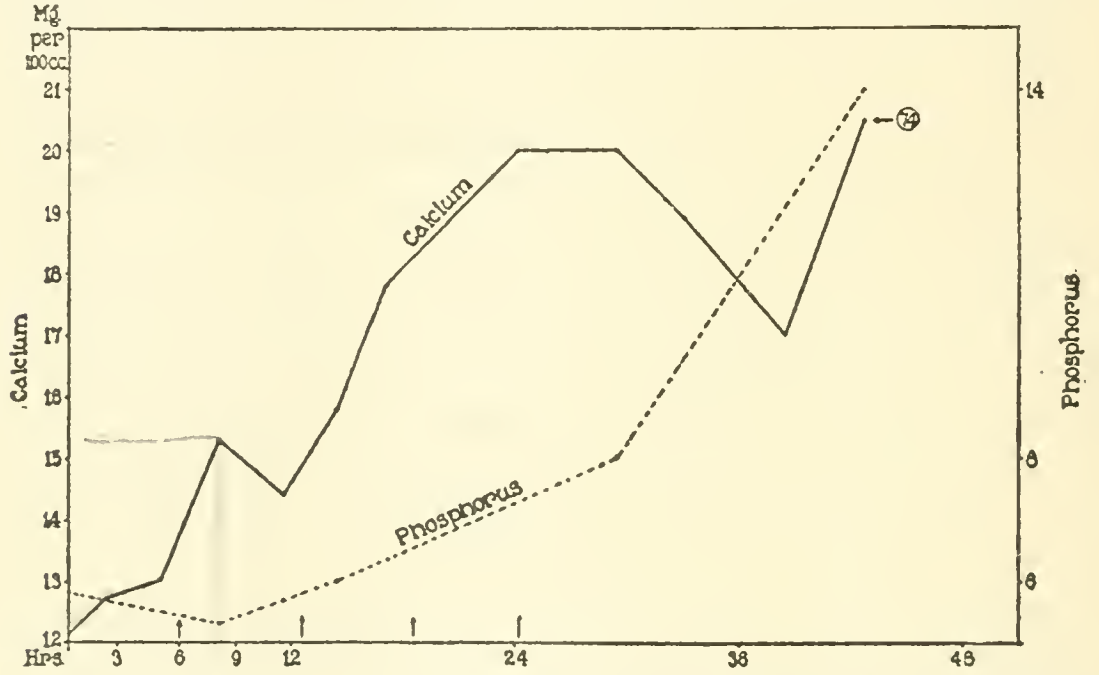

CHART 4. Indicating the blood serum calcium and the whole blood inorganic phosphorus curves, in parathyroid overdosage due to pyramiding in the normal dog.

hours) the blood calcium curve is steadily rising at a uniform rate. The peak point of the blood calcium curve is reached at about 20 mgm. per 100 cc. It is maintained at this level for some hours and then the blood calcium starts to fall. The animal meanwhile may continue to have occasional attacks of vomiting and diarrhea, and is physically becoming more and more depressed. A certain degree of respiratory distress may also be noted. Coincident with the fall in the blood calcium curve urgent symptoms become manifested. There is vomiting and passing of blood by bowel and the animal passes into a state of collapse. Death follows as a rule within a few hours. In this

period of urgent symptoms the blood phosphorus (inorganic) rises abruptly. The blood urea and non-protein nitrogen also increase several hundred per cent. There is a marked decrease in blood volume and a characteristic thickening of the blood. The coagulation time is decreased. The circulation gradually fails and blood samples are obtained from peripheral veins only with great difficulty. The car-

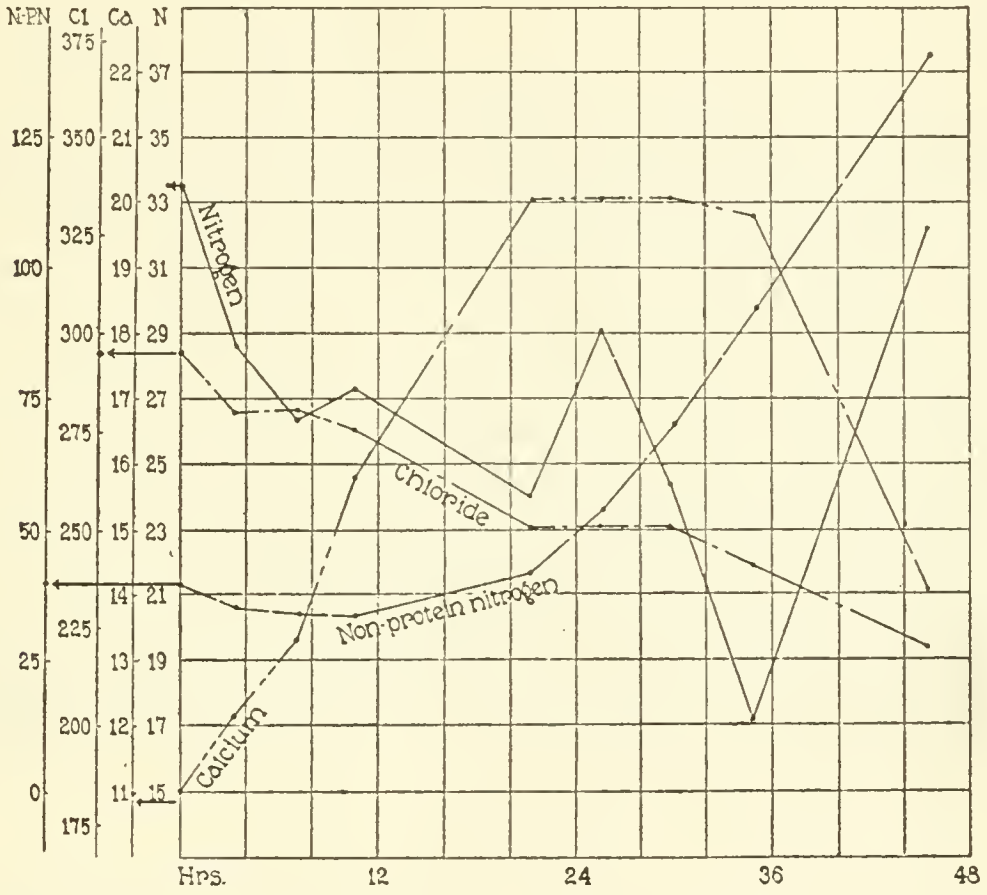

CHART 5. The blood serum calcium, whole blood chloride, nitrogen, and non-protein nitrogen, as affected by repeated injections of 25 units of the hormone and frequent bleedings, in the normal dog.

bon dioxide content and combining power of the blood serum are as a rule definitely and gradually increased during the first half of such experiments. The pH of the blood serum as determined by the colorimetric method of Cullen may be coincidently increased very slightly. This would indicate that in this period there is a tendency towards alkalosis which is however well compensated. The increase in CO_2 content of the blood serum is maintained for several hours, then

this value gradually decreases and in the terminal state is greatly reduced. The pH on the other hand remains stationary until within a few hours of death when it decreases very rapidly. The general effect therefore of parathyroid hormone overdosage upon the acid-base balance is to produce a condition of compensated alkalosis on the first day, this then passes over into a condition of compensated acidosis which in turn is followed by an uncompensated acidosis just prior to death.

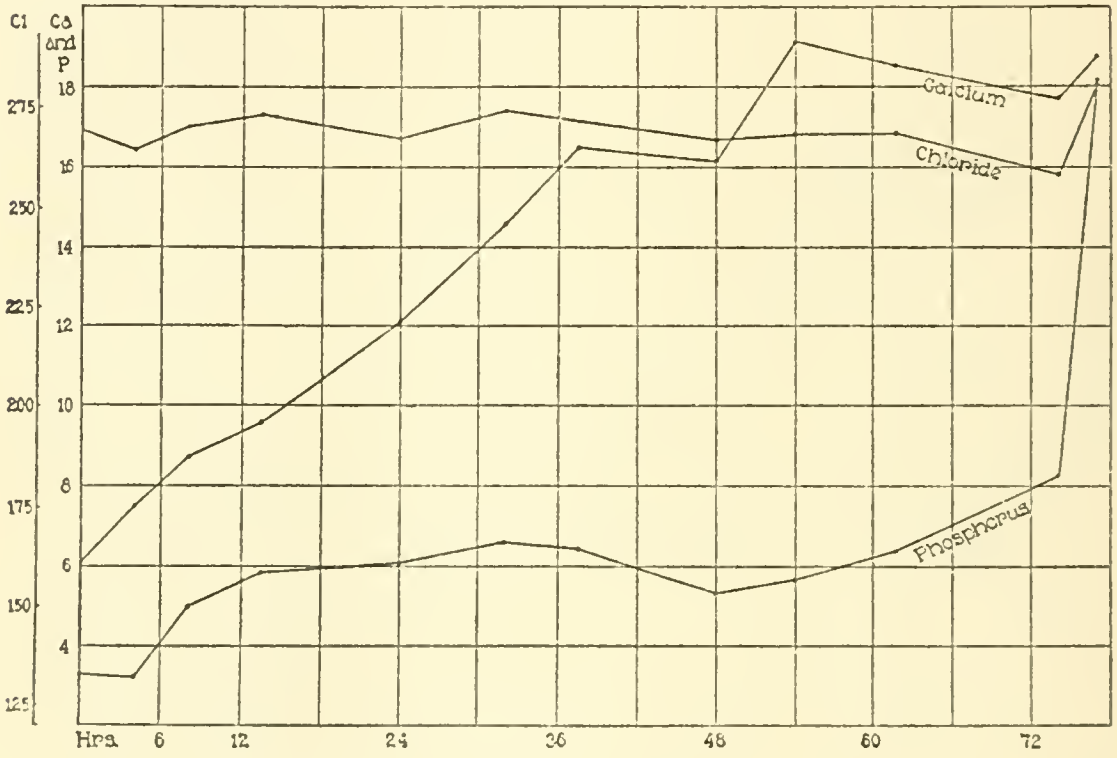

CHART 6. The blood serum calcium and whole blood inorganic phosphorus and chlorides as affected by repeated injections of 25 units of the hormone in a thyroparathyroidectomized dog.

The urinary findings in parathyroid hormone overdosage are also of interest. The kidney practically ceases to function very abruptly at about the time that the serum calcium curve has reached its peak point. There is a sudden decrease in the volume of urine produced and, as a rule, both a relative and an absolute decrease in the rate of excretion of phosphorus, ammonia and titratable acid. Coincident with this abrupt decrease in kidney function the curves for whole blood phosphorus, urea, and non-protein nitrogen start to ascend.

These changes and their time relationships are best illustrated by an inspection of the curves in the text.

Post-mortem examination of a dog dying from parathyroid hormone overdosage discloses marked congestion of the alimentary canal and the presence of more or less blood in the stomach and intestine.

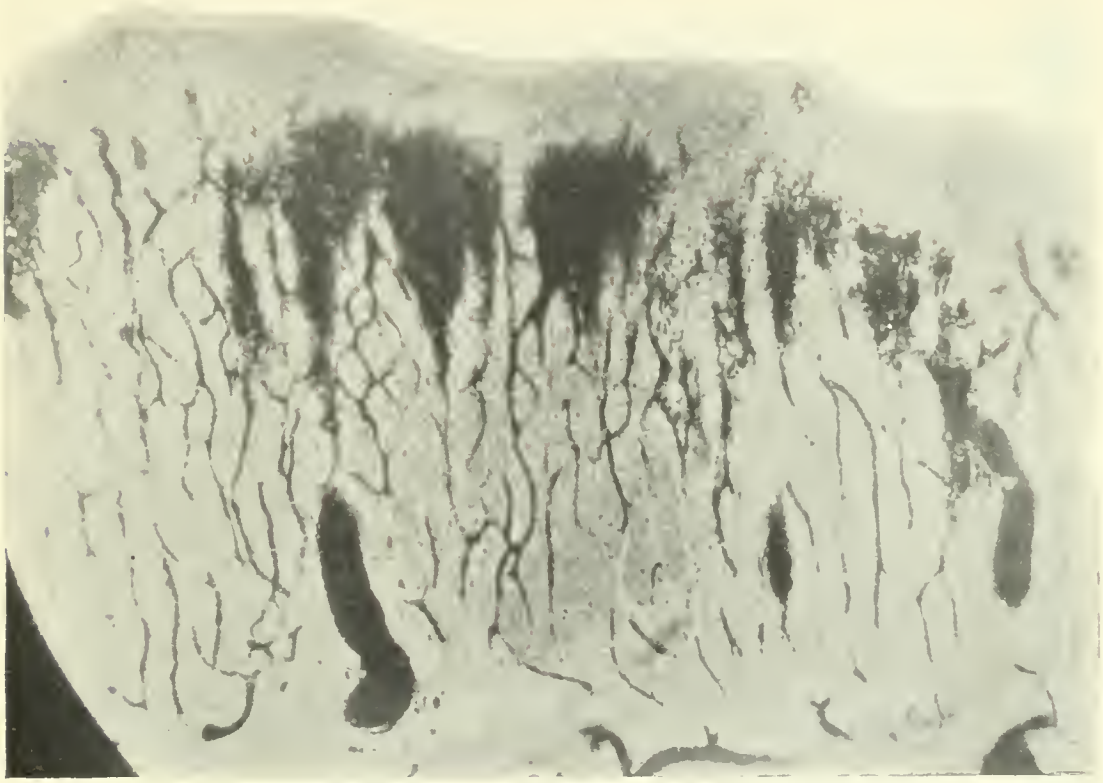

FIG. 2. PHOTOMICROGRAPH OF MUCOSA OF STOMACH OF DOG DYING FROM PARATHYROID HORMONE OVERDOSAGE

Note the dilated capillaries and venules, also the hemorrhagic areas. Section prepared by courtesy of Dr. R. F. Shaner.

Dr. R. Shaner of the department of anatomy of this University has very kindly examined microscopic sections from the stomach of such animals (fig. 2) and also of animals dying from injections of calcium chloride and acid sodium phosphate (fig. 3). He has described these sections as follows:

Sections from the stomach will show a marked change in the blood vessels in the tunica propria, between the gastric glands and gastric pits.

As Mall has shown, a fine capillary plexus surrounds the gastric glands.

It is fed by arterioles which arise from the submucosal arteries, penetrate the muscularis mucosa and end in the deep part of the capillary plexus. The plexus is drained by small veins which arise superficially between the gastric pits, just beneath the inner surface of the stomach. The veins run back through the tunica propria and muscularis mucosa into the submucosa.

In the stomach of the dog dead from an overdose of parathyroid extract the capillary plexus around the glands, and its veins are moderately dilated,

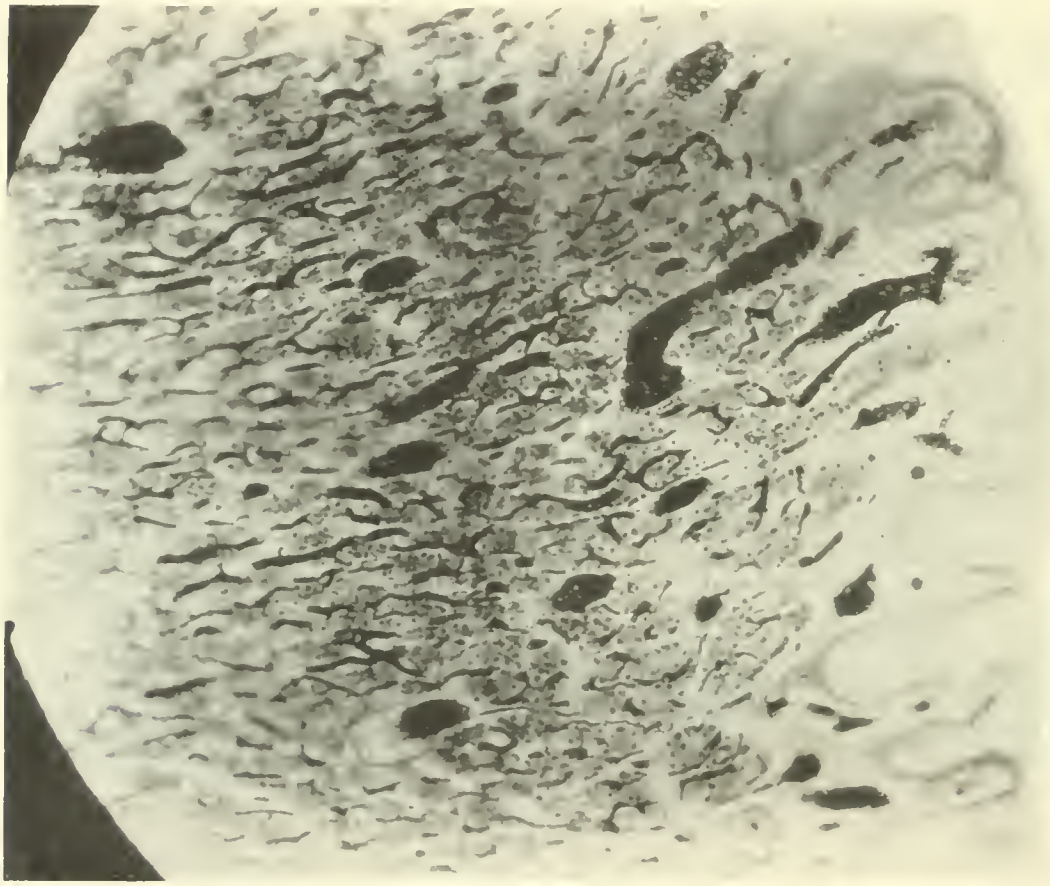

FIG. 3. PHOTOMICROGRAPH OF MUCOSA OF STOMACH OF DOG DYING FROM $CaCl_2$ AND NaH_2PO_4 INJECTIONS

Contrast with figure 2. Section prepared by courtesy of Dr. R. F. Shaner

more so superficially where the capillaries pass into the veins. In this superficial zone there is also a general extravasation of blood from the ends of the capillaries and the first parts of the veins into the surrounding tunica propria. There seems to be no actual rupture of endothelial walls, for these can be traced through the cell masses. There is no special dilation of either veins or arteries in the submucosa. Apparently a general diapedesis takes place. The escaping blood first fills the tissue between the gland pits and then seeps out into the stomach cavity.

The stomach of the dog killed by overdose of calcium and phosphorus shows much the same conditions. The capillary-plexus is even more dilated, being affected in the deep as well as in the superficial parts. In the area from which the section was taken no bleeding had occurred, hence no extravasation of blood appears in the sections. There is no doubt, however, that this took place elsewhere, and in the same manner as in the dog killed by parathyroid extract.

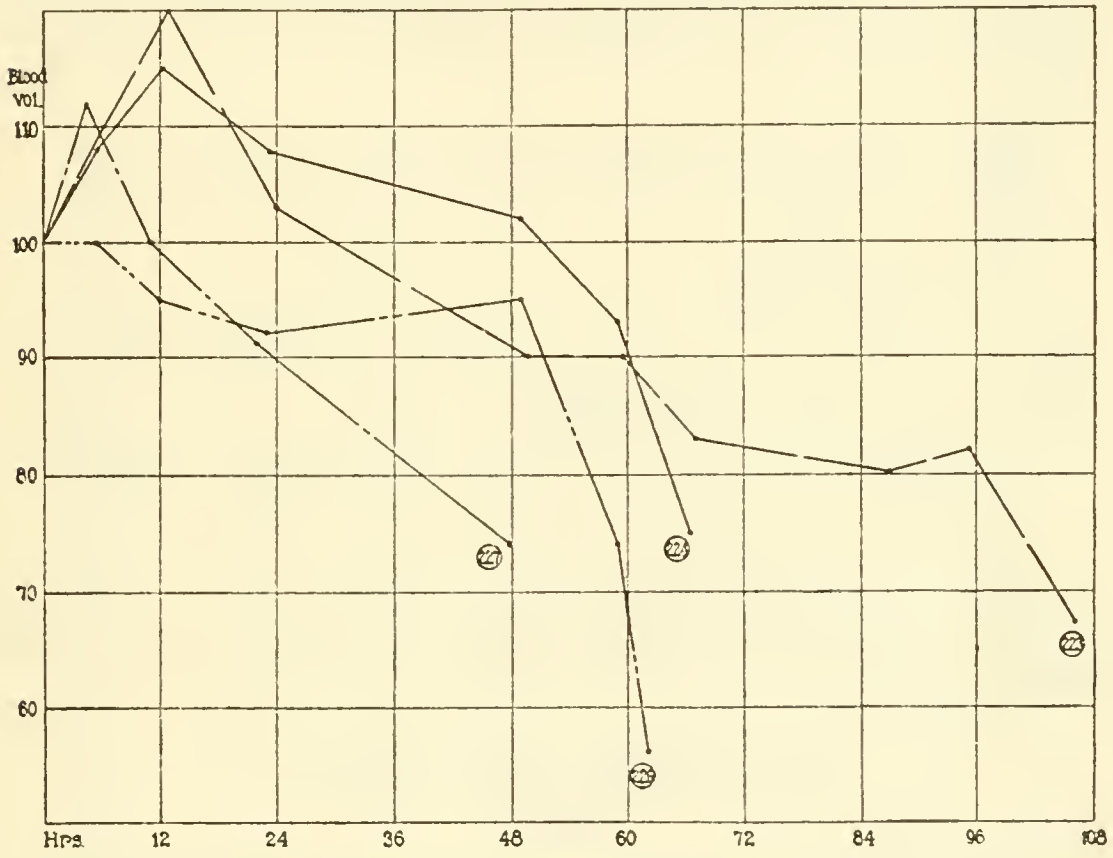

CHART 7. Showing the effect of repeated injections of 25 units of the hormone upon the blood volume as determined by estimation of hemoglobin content.

Rabbits and rats

It had been our experience that the normal rabbit and rat are immune to repeated injections of the hormone and indeed showed very little change in blood serum calcium values under such treatment. Macleod and Taylor have obtained similar results. Recently, however, we have shown that hypercalcemia can be induced in most normal rabbits if enormous dosage of the active extract is administered either in single injections or in repeated doses. No effect on inorganic

phosphorus of whole blood was noted in these experiments, neither were any signs of overdosage phenomena produced. These observations taken in conjunction with others which will be referred to in detail later on, force the conclusion that hypercalcemia alone is not deleterious. The overdosage phenomena seen in dogs and cats are in our opinion due to a tripod of conditions, namely, hypercalcemia, hyperphosphatemia and in the terminal stage extreme acidosis. The latter state is in all probability induced by the two former conditions.

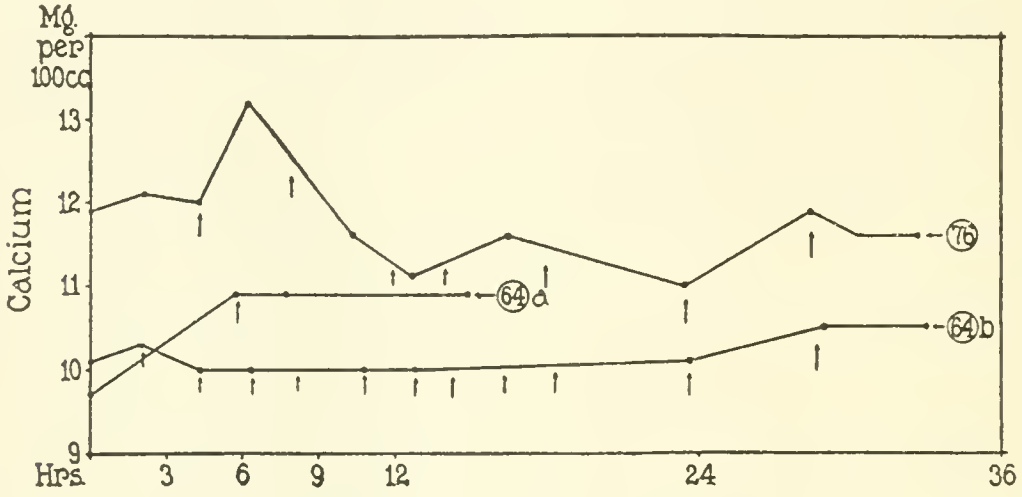

CHART 8. Showing the non-effect of repeated administration *per os* of the para thyroid hormone.

PARATHYROIDECTOMIZED ANIMALS

Dogs

The effect of single injections of the active extract into parathyroidectomized dogs is in no way different from the effect on normal animals. There is the characteristic blood calcium curve following the injection. The quantitative response in blood calcium elevation is of the same order as is found in the normal animal. This is of particular interest on account of its bearing on the possible relationship of the thyroid to parathyroid function. As our parathyroidectomized dogs are thryoidectomized as well, the fact, that the effect of the hormone is, as far as one can judge, quantitatively the same in both normal and thyroparathyroidectomized dogs, argues against any influence antagonistic or otherwise of the thyroid upon the parathyroid glands.

Overdosage phenomena and death are produced in thyroparathyroidectomized dogs by repeated injections of the extract just as in the case of normal animals and the same characteristic symptoms and findings are noted.

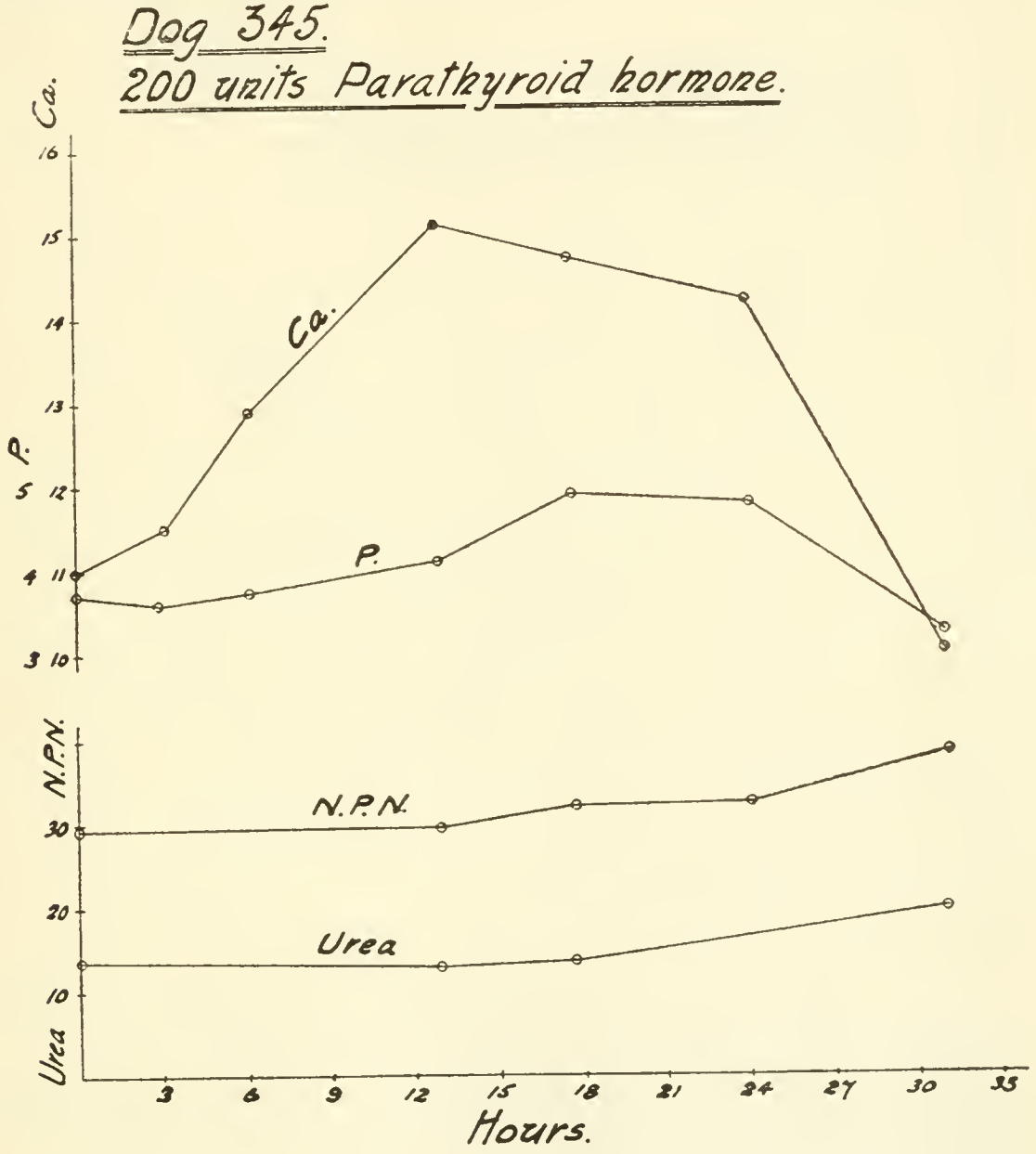

CHART 9. Dog 345, ♂ 25.5 kilos. Showing curves for blood serum calcium whole blood inorganic phosphorus, non-protein nitrogen and urea following single injection of 200 units of the parathyroid hormone.

If the parathyroidectomized animal is in a state of tetany prior to the injection of the active extract there is, coincident with the elevation in the level of blood calcium, relief from tetany and the restoration

of normalcy. The production of profound hypercalcemia by overdosage results in the typical symptoms of this condition becoming apparent and death ensuing.

The curve of inorganic blood phosphorus in thyroparathyroidectomized animals submitted to overdosage with the parathyroid hormone

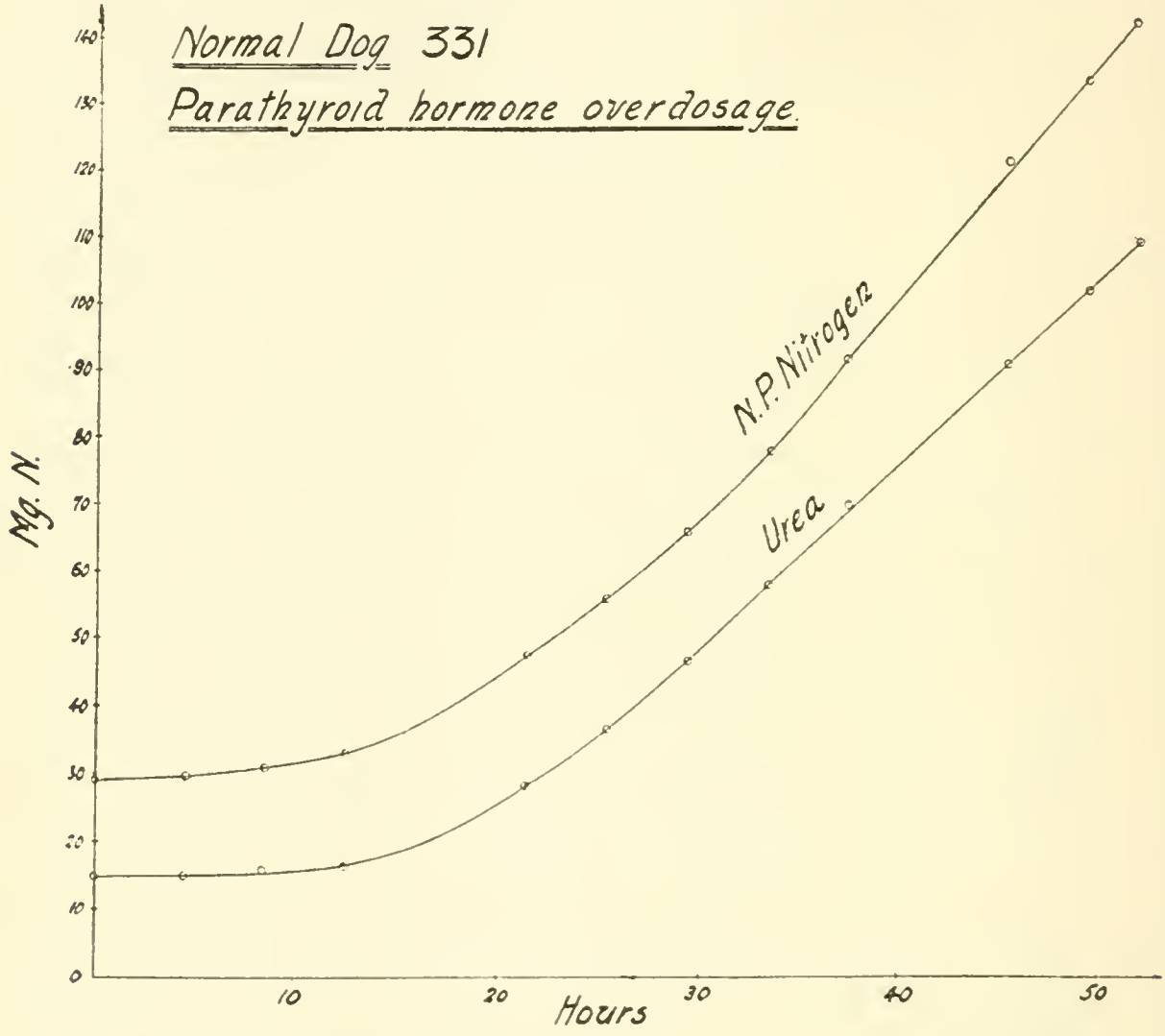

CHART 10. Dog 331, ♂, 18.5 kilos. Showing effect of parathyroid hormone overdosage upon the urea and non-protein nitrogen curves. The curves compare favorably with those for retention. See chart 11.

is of interest. It has been our experience that the inorganic phosphorus of the blood of tetany dogs tends to be elevated as Salveson and others have also observed. The first effect upon the inorganic phosphorus of the blood induced by injections of the hormone in these animals is as a rule, a reduction to lower values, such as 6 or 7 mgm.

reduced to 4 mgm. per 100 cc. If overdosage phenomena are produced, however, the inorganic phosphorus rises in the preterminal period to very high levels just as in the case of the normal dogs so treated. Very recently we had the opportunity of treating a case of post-opera-

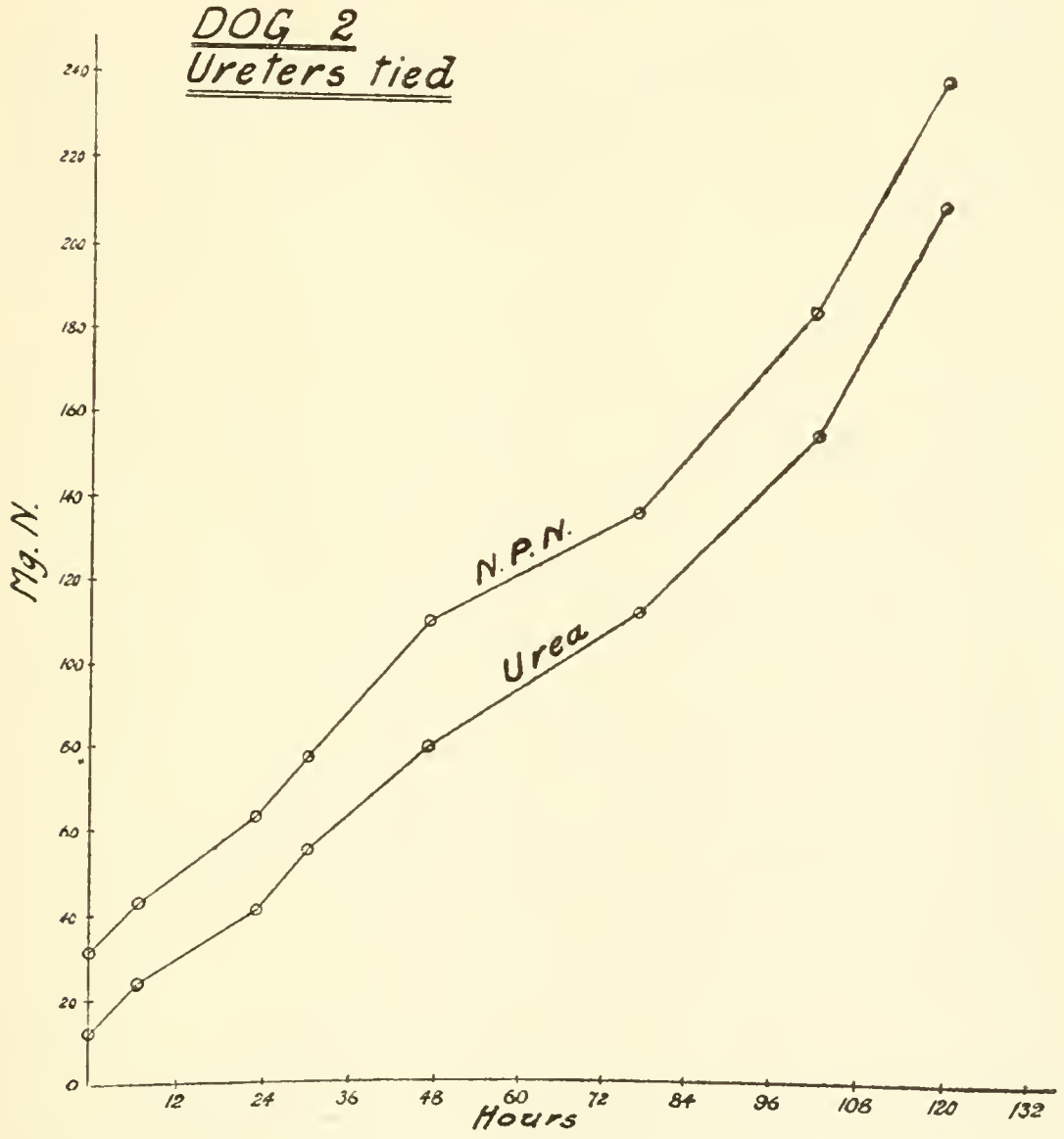

CHART 11. Dog 2, ♀, 16.5 kilos. Both ureters ligated under ether anesthesia. Showing effect of suppression of kidney function upon blood urea and non-protein nitrogen.

tive hypoparathyroidism in a human subject, with the hormone. The blood serum calcium in this case was 5.5 mgm. per 100 cc. and the inorganic phosphorus of the whole blood was 7.1 mgm. per 100 cc. prior to the use of the active extract. In the absence of calcium

CHART 12. Dog 373, ♂, 14.5 kilos. Forty cubic centimeters of 5 per cent $CaCl_2$ injected intravenously followed immediately by 60 units of parathyroid hormone subcutaneously. Curves show subsequent effect upon blood serum calcium and whole blood inorganic phosphorus. Note the reinforcing action of the calcium lactate per os upon the calcium curve.

therapy the blood serum calcium was raised to 9.4 mgm. and the blood phosphorus reduced to 4 mgm. per 100 cc. with complete relief of symptoms.

Cats

No detailed studies have been made upon parathyroidectomized cats treated with the active extract. Tetany may be prevented or relieved in such animals and the level of blood calcium is raised by the use of the hormone. Overdosage phenomena are similar to those observed in dogs and the terminal rise in blood phosphorus is quite as characteristic.

Rabbits

In view of the fact that normal rabbits are immune to overdosage phenomena and since they show such a sluggish response in the elevation of blood serum calcium following injections of the parathyroid hormone, it is a matter of great interest to study the blood chemistry of these animals as affected by parathyroidectomy and also to observe the effect of the hormone on such parathyroidectomized animals. Such a study has just been completed and the results are very instructive. Removal of the parathyroid glands from normal rabbits has resulted in our experience in the early precipitation of violent tetany, with death following very quickly thereafter. The blood serum calcium starts to fall within a few hours of the operation. During this early period no appreciable changes in blood phosphorus (inorganic) are noted. The onset of fatal tetany is as a rule very rapid. The majority of our untreated animals died within thirty hours. Some hours previous to the onset of tetany and some hours after the blood calcium has started to fall the inorganic phosphorus (as determined by the Briggs method) rises markedly and in the tetany stage reaches very high levels. After tetany is manifested death occurs very rapidly as a rule and injections of parathyroid hormone or even of calcium chloride may be of little avail. The most striking change in the blood chemistry induced by parathyroidectomy in rabbits is the enormous rise in blood phosphorus in the preterminal stage. In this particular these animals differ most from dogs and herein is probably the explanation for the violent and unyielding character of the

tetany manifested in these animals. These observations tend to emphasize the importance of phosphorus in relation to the pathogenesis of tetany, a fact which Greenwald has long maintained to be of great importance.

The treatment of parathyroidectomized rabbits by the injection of the parathyroid hormone can be successfully carried out in the majority of cases providing the injections are started immediately after the operative removal of the glands. A number of parathyroidectom-

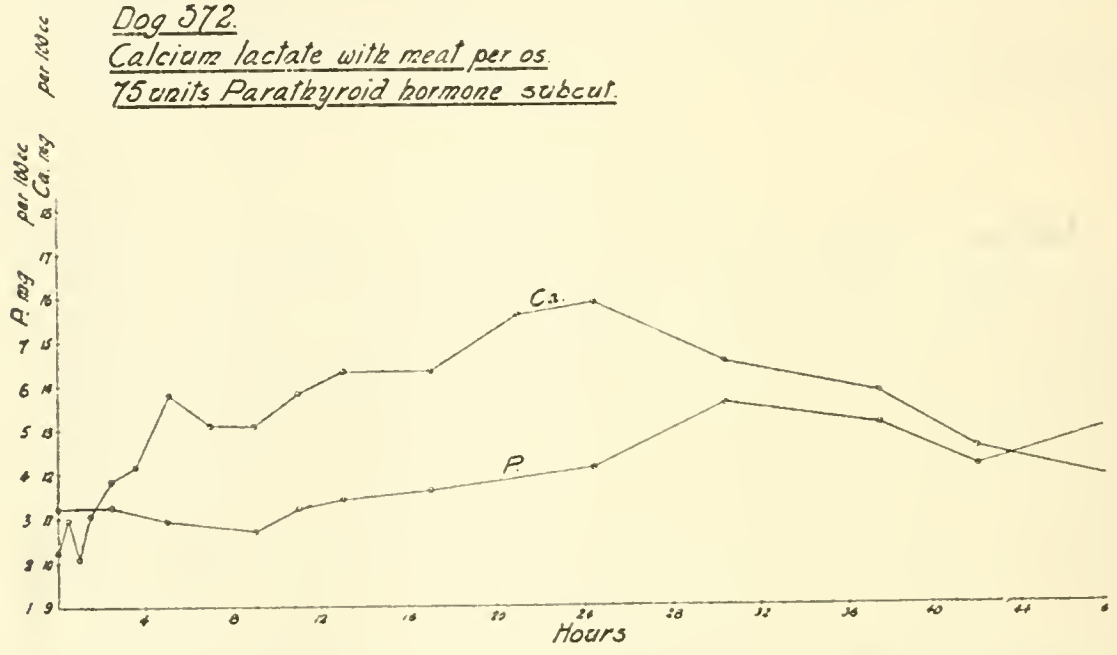

CHART 13. Dog 372, ♂, 16.5 kilos. Seventy-five units of parathyroid hormone injected subcutaneously. Ten grams of calcium lactate fed with lean meat every hour for five hours. Curves show effect upon blood serum calcium and whole blood inorganic phosphorus.

ized rabbits have, however, died during such treatment and at death the blood serum calcium is as a rule only slightly reduced, but the blood phosphorus is found to be very high. Whether such a result is due to too much extract or to an independent break away, so to speak, of the inorganic phosphorus is not yet determined. Certainly by the use of the extract the blood phosphorus may be kept low and the calcium within normal limits in the majority of instances as in the case of parathyroidectomized dogs and cats treated with the hormone.

IN WHAT FORM DOES CALCIUM EXIST IN THE BLOOD?

As not all the calcium in the blood will dialyze, it has been argued that a certain part of it is organically bound. Cameron and Moorehouse (1925) have very recently published results which indicate the existence in the blood plasma of a specific organic compound of calcium. The findings of Cameron and Moorehouse are of great significance and will be briefly enumerated. Assuming that the cerebrospinal fluid is representative of that part of the plasma which can diffuse through an animal membrane, it is shown that the diffusible calcium of normal dog's plasma averages 53 per cent of the serum calcium. Plasma and serum calcium values of normal dogs are usually equal. Corpuscles are practically calcium free (Kramer and Tisdall (1922), Rona, Petow, and Wittkower (1924)). It is suggested therefore, that 53 per cent of the blood calcium exists in the inorganic form and 47 per cent in organic combination. It is further suggested that in the process of clotting the organic compound of calcium is changed to another organic form which dissociates to a greater extent so that a larger percentage of the serum calcium is diffusible. Removal of the parathyroid glands results in a decrease of the total blood calcium, and of both fractions. There is an apparent gradual increase in the proportion of plasma calcium that can diffuse so that finally, in acute tetany the figures for serum calcium and cerebrospinal fluid calcium tend to become exactly equal. This increase they state, is, however, only apparent since after parathyroidectomy the organic calcium compound with its calcium is more and more completely taken up by the clot so that in acute tetany the serum calcium is less than the plasma calcium by 3 or 4 mgm. per 100 cc. This fact, if confirmed, that the plasma calcium and therefore the whole blood calcium, is only slightly reduced in acute tetany while the serum calcium is reduced by approximately 50 per cent of its normal value would seem to throw a new light on the nature of the blood calcium and also on its rôle in parathyroid tetany. Cameron and Moorehouse find that the decrease in whole blood calcium induced by parathyroidectomy is due to a decrease in both the diffusible and nondiffusible forms. The conception of a series of interlocked equilibria between calcium ions and undissociated inorganic calcium compounds

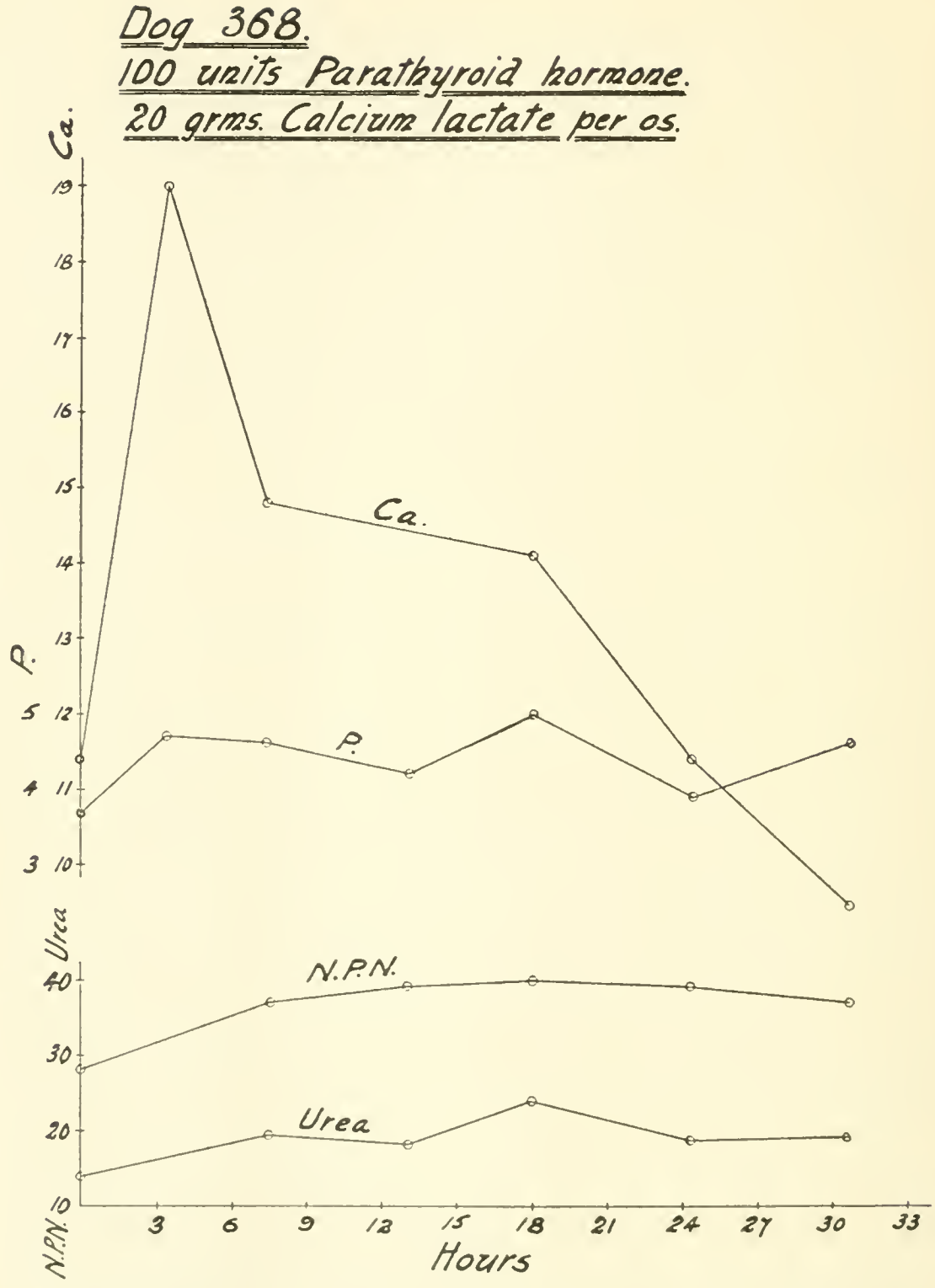

CHART 14. Dog 368, ♀, 17.5 kilos. Showing curves for blood serum calcium, and whole blood inorganic phosphorus urea and non-protein nitrogen following single injection of 100 units of the parathyroid hormone. Ten grams of calcium lactate were administered by stomach tube at beginning of experiment and again at 3.7 hours.

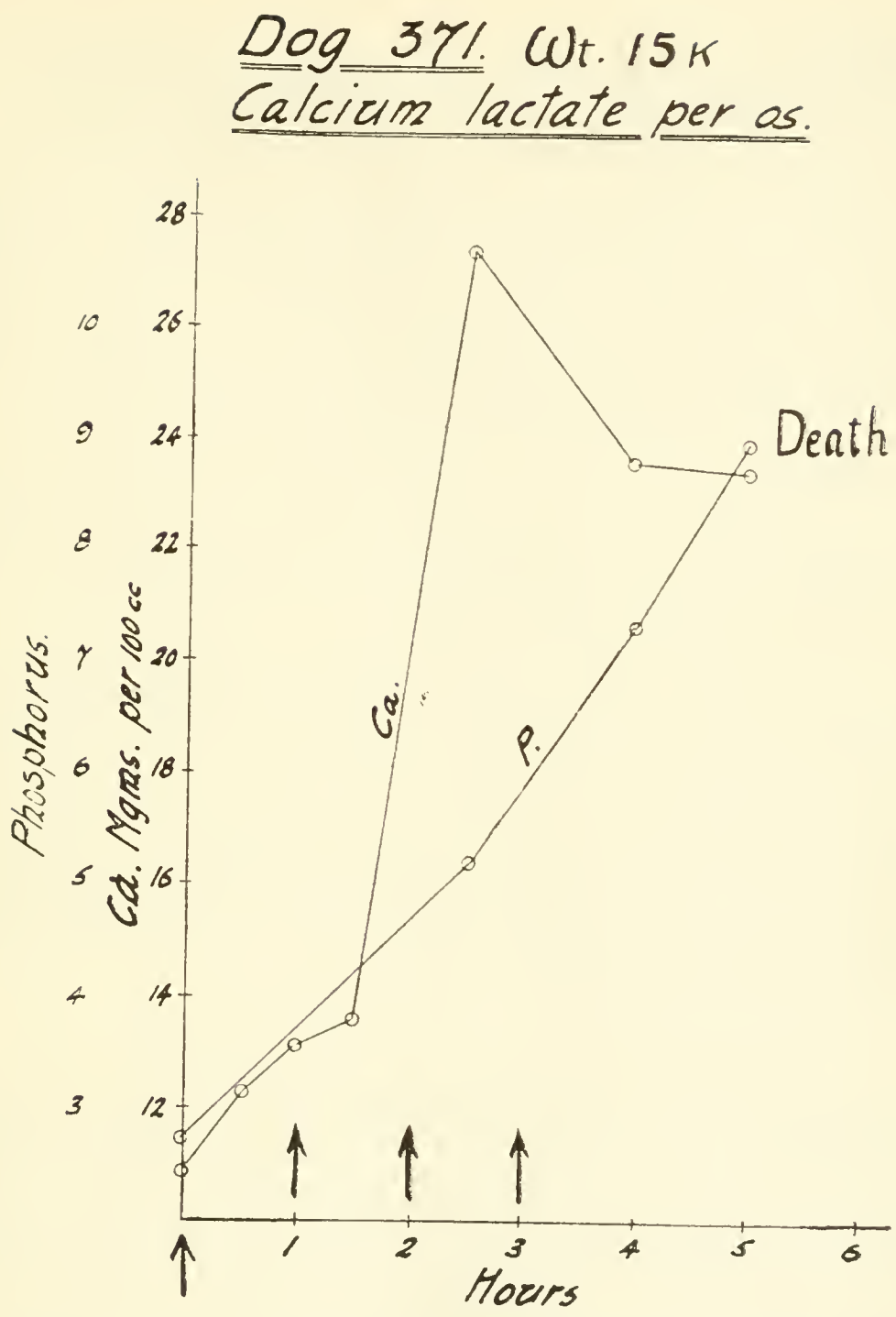

CHART 15. Dog 371, ♀, 15 kilos. Ten grams of calcium lactate given by stomach tube at times indicated by arrows on chart. Profound hypercalcemia followed and death ensued. The blood became very viscous and it was difficult to separate the serum therefrom.

and organic calcium (CaX) is presented. Tetany is due to a decrease in the inorganic calcium of the plasma resulting indirectly from a diminution of the organic compound. In testing out the activity of the parathyroid hormone on parathyroidectomized dogs, Cameron and Moorehouse (1925) report as follows: "After injection into the parathyroidectomized animal the serum and plasma values (calcium) rise and tend to become equal, the whole blood calcium increases correspondingly and the cerebrospinal fluid shows a delayed rise."

That the organic calcium compound is entirely convertible into the inorganic form is shown by the fact that ammonium oxalate added to serum results in the quantitative precipitation of the serum calcium. This is only so, however, when sufficient time is allowed to elapse before the recovery of the calcium as the calcium oxalate precipitate is attempted. Such a result is entirely in accord with the theory advanced by Cameron and Moorehouse.

Attention has been focused on the importance of ionized or ionic calcium in the blood (Vines (1921)). No one will dispute the great importance of "ionic" calcium values but until some method is devised which will permit of the accurate determination of the calcium ion-concentration in blood and body fluids, little progress can be made in this field. It is the opinion of the writer that the absolute concentration of calcium ions is the most important factor in calcium metabolism and that this factor is normally regulated either directly or indirectly by the parathyroid glands.

THE ESTIMATION OF BLOOD SERUM CALCIUM

Excepting blood phosphorus (inorganic) the determination of the calcium content of the blood serum is the only analytical procedure necessary in studying the effects of parathyroid therapy either in man or animals. There are many chances of error in this determination and to such an extent is this the case that many published reports on blood calcium are valueless because the various possibilities of error have not been eliminated. The method which has been found most satisfactory in our laboratory is a purely empiric one being a slightly modified Tisdall-Kramer procedure (Clark and Collip (1925)). In order that uniform and entirely satisfactory results may be obtained it is necessary to adhere most rigidly to the prescribed tech-

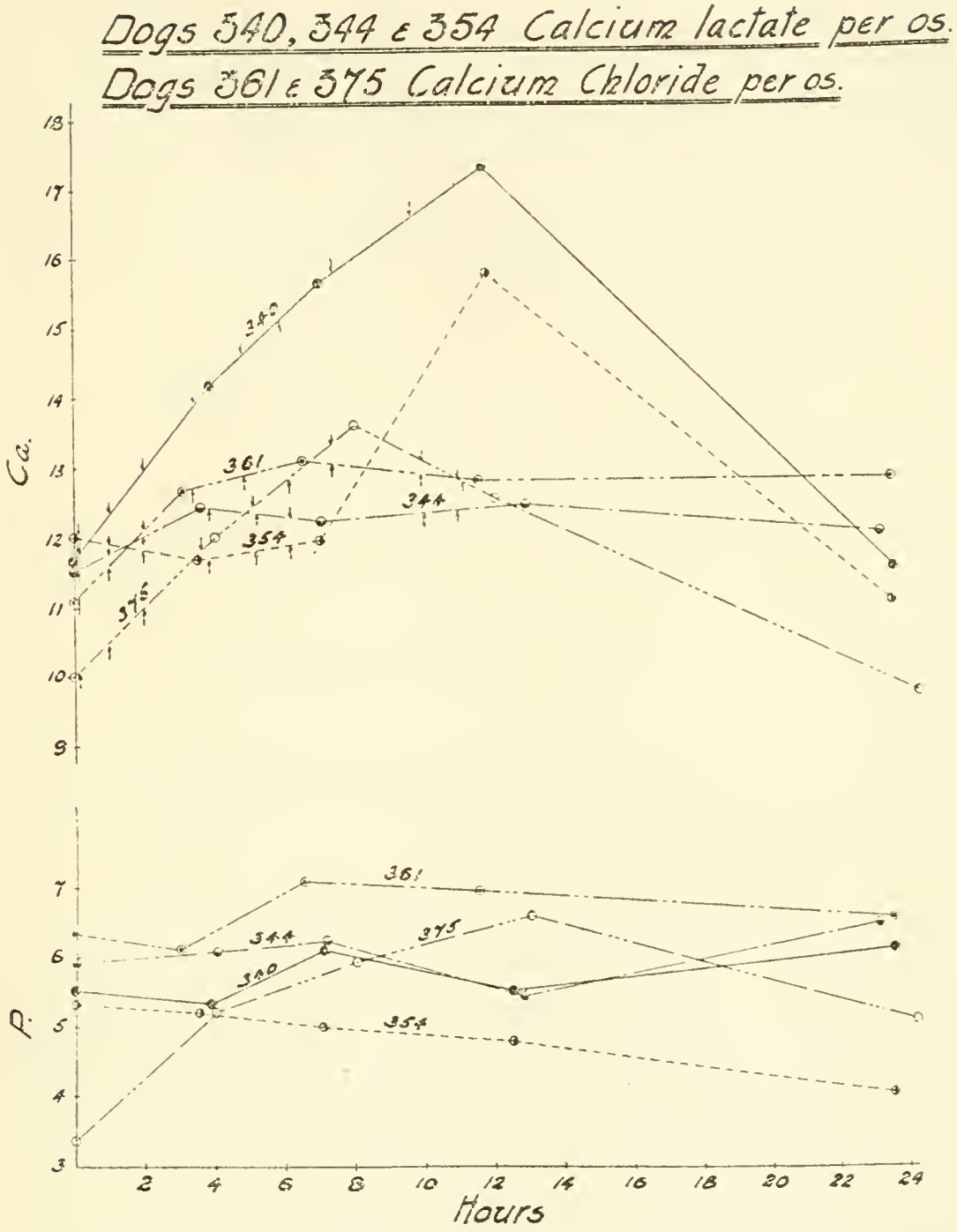

CHART 16. Dog 340, ♂, 18.5 kilos. Dog 344, ♂, 12.5 kilos. Dog 354, ♂, 18 kilos—thyroparathyroidectomized. Dog 361, ♂, 10 kilos. Dog 375, ♂, 11 kilos. Dogs 340, 344, 354, received 10 grams calcium lactate in 500 cc. of water at times indicated on chart. Dogs 361 and 375 received 5 grams of calcium chloride in 400 cc. of water at times indicated on chart.

nique. The manner of handling the blood specimen is also of equal importance. Care should be observed to separate the serum from the sample, which has been allowed to clot spontaneously, as soon as possible. Also, it is most important that the sample should not be chilled (as by being temporarily placed in an ice chest). If the sample cannot be dealt with at once it may be allowed to stand at room temperature for a few hours. Chilling of the sample causes a definite decrease in the serum calcium value.

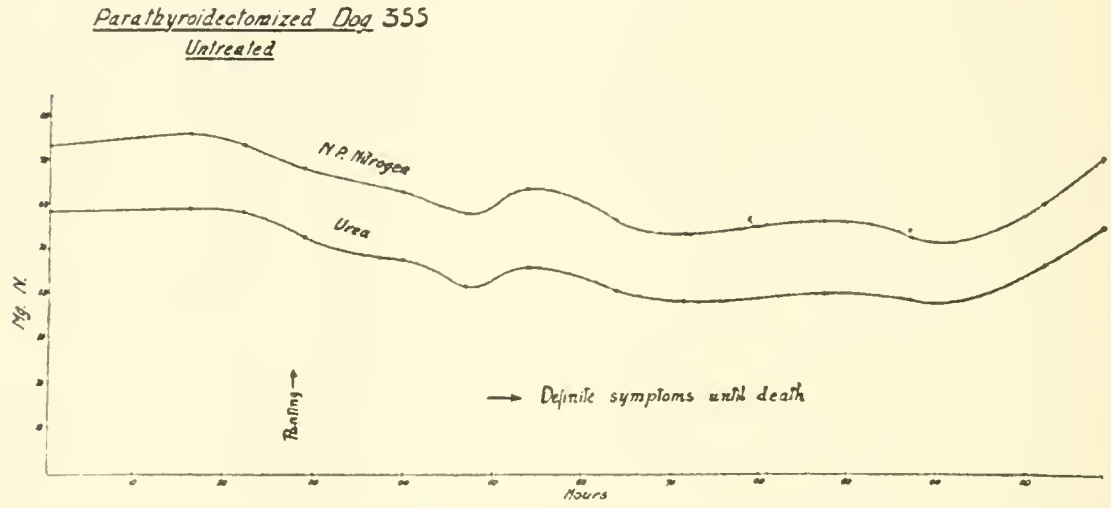

CHART 17. Dog 355, ♂, 22.5 kilos. Showing effect of thyroparathyroidectomy upon the blood urea and non-protein nitrogen.

THE RÔLE OF GUANIDINES IN PARATHYROID TETANY

The writer is of the opinion that guanidine intoxication bears no relationship to parathyroid tetany. It is admitted that Paton and his collaborators have made out a strong case for such a relationship and that this theory cannot be summarily dismissed. There is now, however, so much evidence which is not in accord with the guanidine intoxication theory that it is felt that this theory is no longer tenable.

It has been claimed that the injection of guanidine causes a decrease in blood calcium (Watanabe, Sharpe, Gyorgy and Vollmer). Nelken and Salveson have failed to confirm this as have also Collip and Clark.

Collip and Clark have observed guanidine intoxication in dogs coincident with parathyroid hormone overdosage. No antagonism was demonstrated in these experiments between the parathyroid hormone and guanidine.

Vines (1923) devised a method of estimating the degree of activity of parathyroid preparations which would appear to depend on the degree to which guanidine is destroyed. White and Cameron (1925) have recently applied Vines' method to known potent parathyroid extracts and to certain other substances. They summarize their results as follows:

Vines' method for estimating the activity of parathyroid preparations shows negligible activity when used with Collip's active extract.

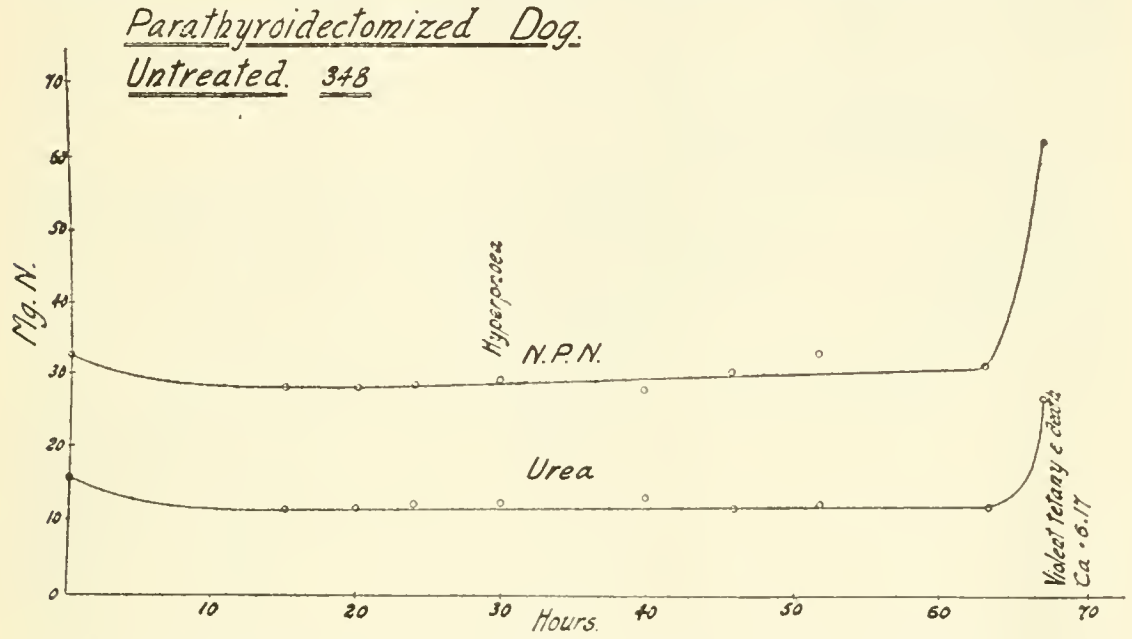

CHART 18. Dog 348, ♂, 24 kilos. Showing the effect of thyroparathyroidectomy upon the urea and non-protein nitrogen of the blood.

Since it appears to depend on the inhibiting influence of complex substances such as arginine or nucleic acid derivatives on the precipitation of guanidine picrate, such results as have been obtained by Vines for animal tissues (using this method) probably bear no relation to the function of those tissues.

In a recent communication Collip and Clark (1925) report on the nature of the urea and non-protein nitrogen curves for the blood of untreated parathyroidectomized dogs. It was found that the urea and non-protein nitrogen curves run practically parallel throughout an experiment. Just before death occurs, there may be slight deviation of the curves. The curves fail to show any evidence of the accu-

mulation of non-protein nitrogen containing compounds (such as guanidine) after parathyroidectomy. Salveson (1923) found the non-protein nitrogen and urea normal during tetany. Recently we have observed normal values for urea and non-protein nitrogen in a most severe case of post-operative hypoparathyroidism.

Normal dogs were also injected with guanidine compounds and the urea and non-protein nitrogen curves for the blood were determined. These curves by contrast illustrate when sufficient guanidine has been administered to produce symptoms that a marked increase in the non-protein nitrogen of the blood has occurred. The general character

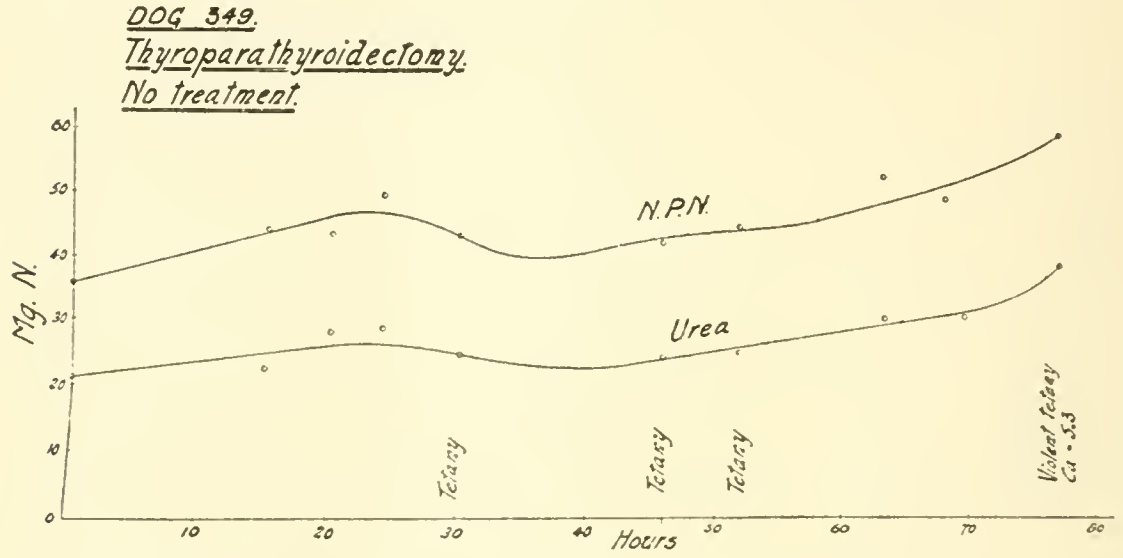

CHART 19. Dog 349 ♂, 16 kilos. Showing effect of thryoparathyroidectomy upon the blood urea and non-protein nitrogen.

of these urea and non-protein nitrogen curves is such as to indicate that the phenomena of guanidine poisoning is quite a different thing from parathyroid tetany.

Such evidence taken in conjunction with Greenwald's failure to demonstrate a toxin in the blood of parathyroidectomized dogs in a state of tetany would seem in our opinion to warrant the dismissal of the guanidine hypothesis.

LATENT TETANY

This condition was first described by Vassale (1898). A dog from which three parathyroid glands had been removed, showed transitory

symptoms of tetany from which it completely recovered. Eighteen months after the operation the animal gave birth to eight puppies and during the lactation period five days later had a violent attack of tetany.

Luckhardt and Blumenstock (1922) observed the recurrence of acute tetany in completely parathyroidectomized dogs during the

FIG. 4. COMPLETELY PARATHYROIDECTOMIZED DOG THIRTEEN MONTHS AFTER OPERATION

Animal has been kept on a lean meat diet. Fifteen units of parathyroid hormone daily are sufficient to offset tetany. Withdrawal of the extract has at any time resulted in the early precipitation of tetany.

period of oestrus. These animals had been cured of all symptoms of tetany by the intravenous injection of Ringer's solution daily for a period of about forty days following the operation.

Luckhardt and Goldberg (1923) kept completely parathyroidectomized dogs alive on a meat diet by the oral administration of massive doses of calcium lactate. They observed that calcium therapy could be withdrawn after several months without the precipitation of tetany.

Spontaneous tetany occurred thereafter at irregular intervals and was rapidly controlled by the oral administration of calcium lactate and the giving of enemas.

Salveson (1923) observed that dogs in a state of latent tetany, but showing no symptoms, months after complete parathyroidectomy still have a low serum calcium and a slightly elevated inorganic phosphorus.

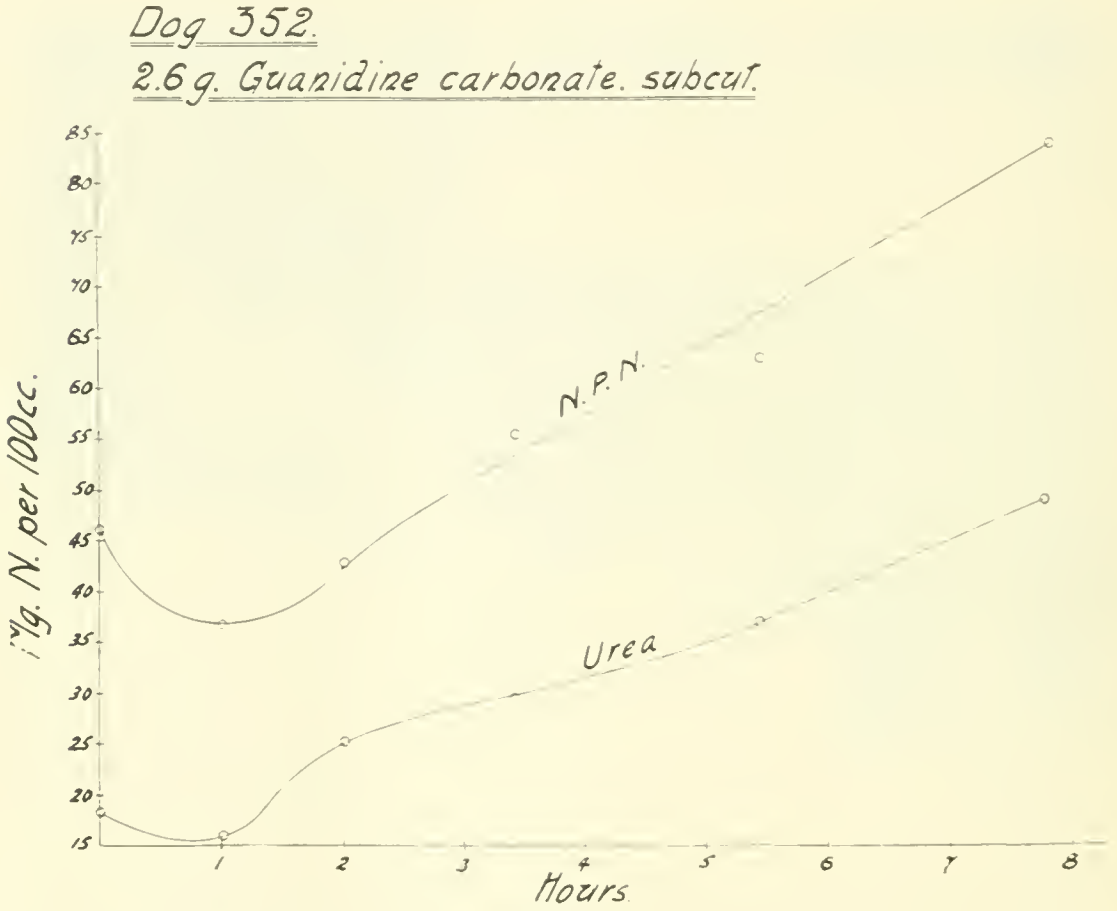

CHART 20. Dog 352, ♂, 17 kilos. 2.6 grams of guanidine carbonate injected subcutaneously.

It has been observed in our own laboratory that certain parathyroidectomized dogs which have been kept in a normal condition for six weeks or more by the daily injection of parathyroid hormone will suffer no immediate ill effects from withdrawal of the treatment. Such animals develop tetany during periods of heat. The blood serum calcium remains at a low level for an indefinite period. Other animals cannot be kept free from tetany under similar dietary conditions (exclusive lean meat diet), unless the treatment is continued.

A rational explanation of these observations is that accessory parathyroid tissue is present in certain instances. Salveson does not accept this view and states that if true parathyroid function were manifested the blood serum calcium should be restored to higher levels. In the light of our own observations that the action of the parathyroid hormone is quantitative, this criticism is not valid. It is the writer's opinion that in true latent tetany parathyroid function is being maintained to a degree and that in the absence of any parathyroid function, tetany on a meat diet is unavoidable. In this latter condition life can only be maintained indefinitely by injections of the hormone or by other therapy designed to maintain the calcium and inorganic phosphorus of the blood within definite bounds (see legend figure 4—dog 48).

THE EFFECT OF DIET AND THE STATE OF NUTRITION UPON THE RESPONSE TO INJECTIONS OF THE PARATHYROID HORMONE

Since dogs and cats are very sensitive to injections of the hormone, and rabbits, guinea pigs and rats are resistant to such injections, it is logical to suppose that the dietary habits of a species might influence the response to the parathyroid hormone. In our own limited experience changing the diet of dogs used in potency testing from lean meat to bread has had very little influence upon the response of these animals to injections of the extract.

Macleod and Taylor have observed that a very fat dog, which responded very sluggishly to the hormone became very sensitive to it after its body fat had been reduced. No doubt further investigation will show that there are many factors which can affect the response to injections of the active extract. The nutritional condition is at least one factor which has this effect.

PARATHYROID HORMONE, ULTRA-VIOLET LIGHT AND THE ANTI-RACHITIC VITAMINE

It is apparent that there is some fundamental relationship between the physiological effects of the parathyroid hormone, of the action of ultra-violet radiations and of the anti-rachitic vitamine. That the action of the former is upon calcium metabolism is proven. It is a possibility that the latter two agents act upon phosphorus metabolism.

It remains however for future research to give an adequate answer to this question.

GENERAL DISCUSSION

The recent preparation and standardization of active parathyroid extracts and the study of their physiological action has confirmed the theory of MacCallum and the more recent work of Salveson on the relationship of parathyroid function to calcium metabolism. Although MacCallum and Voegtlin discovered the favorable effect of calcium in tetany in 1909; it has only been of late that completely parathyroidectomized animals have been kept in a normal state for long periods of time by vigorous calcium therapy. The parathyroidless animal loses the power to maintain calcium in the blood. Salveson has shown that this defect may be overcome by artificially forcing up the level of the blood calcium. He has also demonstrated that this process must be more or less continuous since injected calcium leaves the circulation very rapidly and may be quantitatively recovered in the excreta. Greenwald[4] (1925) finds that the parathyroid hormone administered to normal animals causes an increase in the excretion of calcium. This may be directly due to the increased calcium content of the blood. Calcium injected into the blood stream of the normal animal leaves the circulation very rapidly as Salveson has shown and we have confirmed. It is quite to be expected therefore when the blood calcium is increased in a normal animal by the use of the hormone that there will be an increased excretion of this element. The hormone must be looked upon essentially as a mobilizer of calcium. The native production and liberation of the hormone in the normal animal must be just such as will maintain a dead level of calcium in the blood stream. Since it requires a minimum of 15 units per day to keep a parathyroidectomized animal free from symptoms it is probable that the daily production in the normal human subject would be at least three times this amount.

Recent blood chemistry studies in parathyroidectomized rabbits indicate that the rôle of phosphorus in tetany has not been sufficiently stressed (Collip (1925)). The inorganic phosphorus in this animal

[4] Personal communication.

increases enormously after removal of the parathyroid glands and tetany of the most marked type quickly develops (under thirty hours as a rule), and death follows shortly thereafter. Injections of parathyroid hormone or even of calcium chloride may be of no avail once marked tetany has become manifested. The blood calcium is found to be lowered just as in the case of the parathyroidectomized dog or cat. These animals may still die after the calcium has been raised several milligrams per cent by injections of the parathyroid hormone.

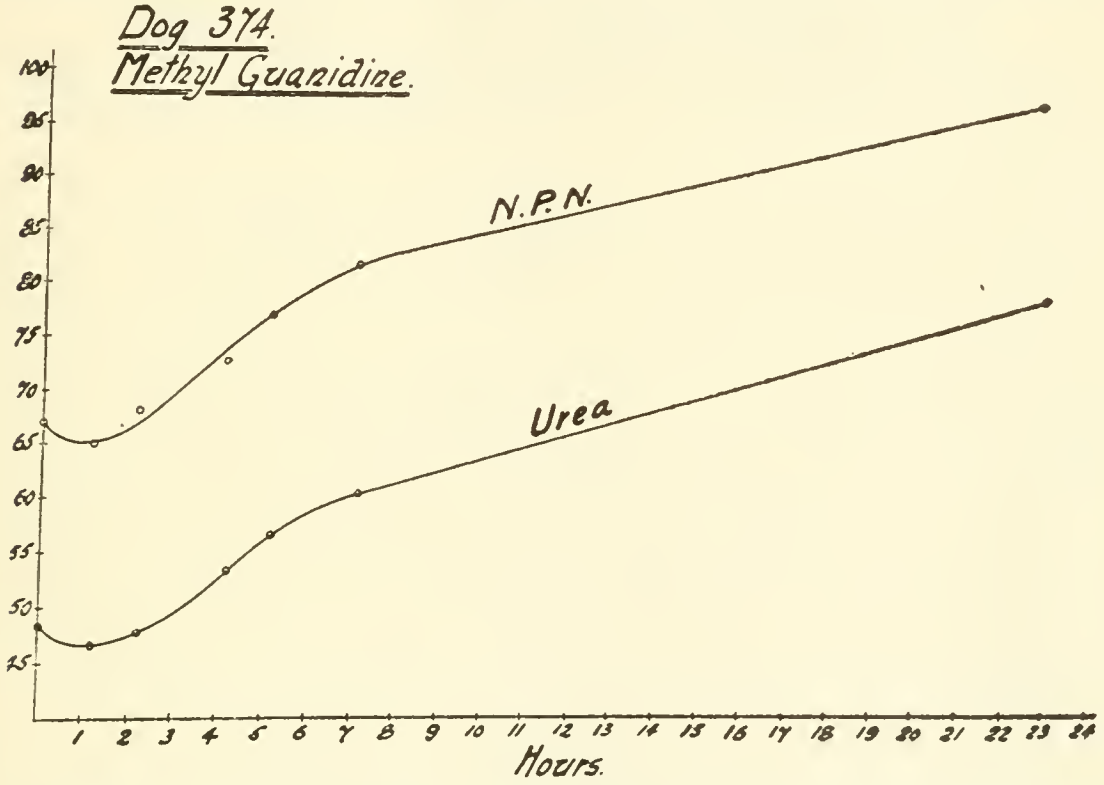

CHART 21. Dog 374, ♂, 20 kilos. Showing effect of the subcutaneous injection of 2 grams of methyl guanidine (sulphate) upon the blood urea and non-protein nitrogen. Death occurred on the third day following the injection.

If treatment with the hormone is instituted immediately after the operative removal of the glands most animals may be maintained in a normal state for some days. Death may occur suddenly, however, in certain of these animals and blood analysis may then show a practically normal calcium but a markedly increased inorganic phosphorus content (20 mgm. per cent). This increase in phosphorus is not due to the extract unless the parathyroidectomized rabbit has some peculiar sensitivity to it which is not seen in the dog, because normal rabbits

are practically immune to any ill effects of parathyroid hormone overdosage. Enormous dosage may cause marked increases in blood calcium values but phosphorus does not change materially. Since the urgent symptoms of overdosage with the hormone seen in dogs and cats are coincident with a falling calcium curve and a rising phosphorus curve, it is probable that these urgent symptoms are due more to the blood phosphorus change than to the high blood calcium. Granted that this is true, then a rise in blood phosphorus would be the natural

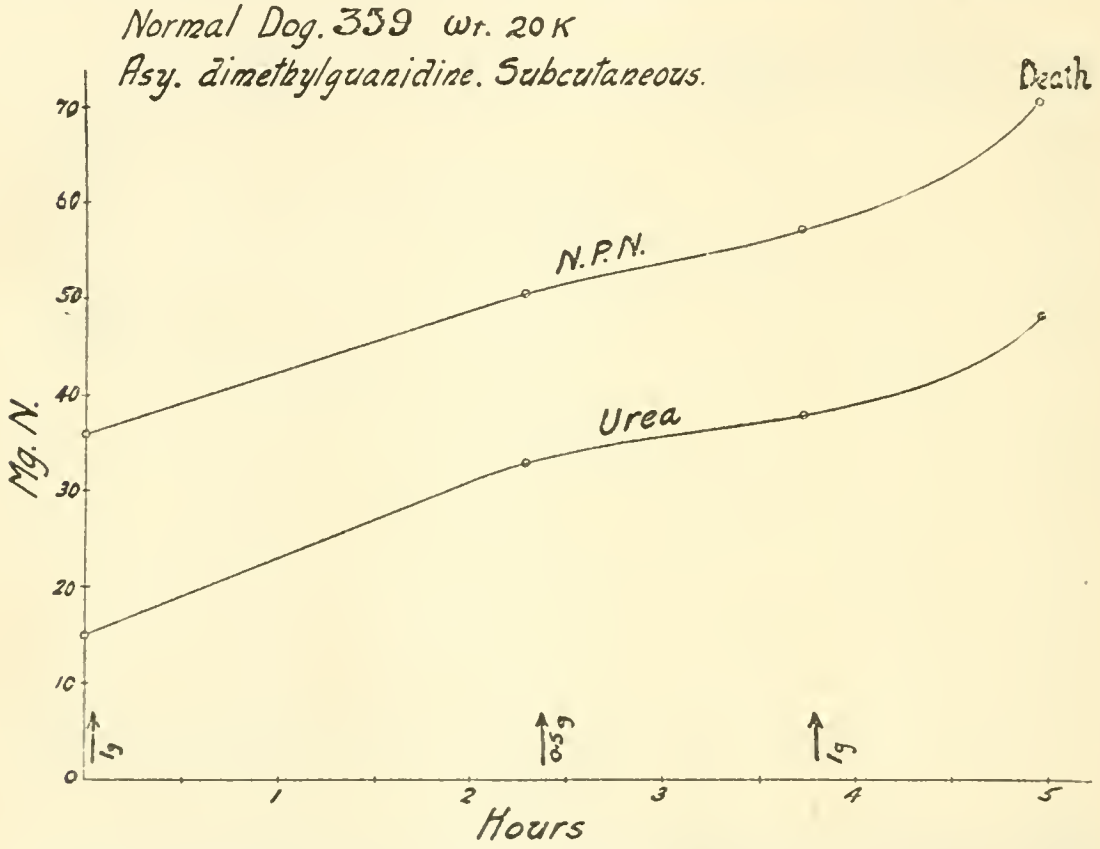

CHART 22. Dog 359, ♂, 20 kilos. 2.5 grams of asymmetric dimethyl guanidine (sulphate) administered as indicated on the chart. Mild tetany was manifested three and one-half hours after experiment was started.

danger signal of overdosage rather than the blood calcium value. It would also furnish a rational explanation of the difference in the toxic effect of the hormone in different animals.

Overdosage phenomena in dogs simulated by means of inorganic salts

In an endeavor to discover the cause of the characteristic phenomena manifested in dogs submitted to overdosage with the parathyroid

hormone, certain inorganic salts have been injected intravenously into normal animals. As a result of this investigation it may be stated that, by injections of calcium chloride and acid sodium phosphate solutions, a condition very similar to that seen in the preterminal state of parathyroid hormone overdosage may be produced. Neither calcium chloride nor sodium acid phosphate solutions alone administered by intravenous injections have produced this effect. Relatively enormous amounts of calcium chloride can be administered

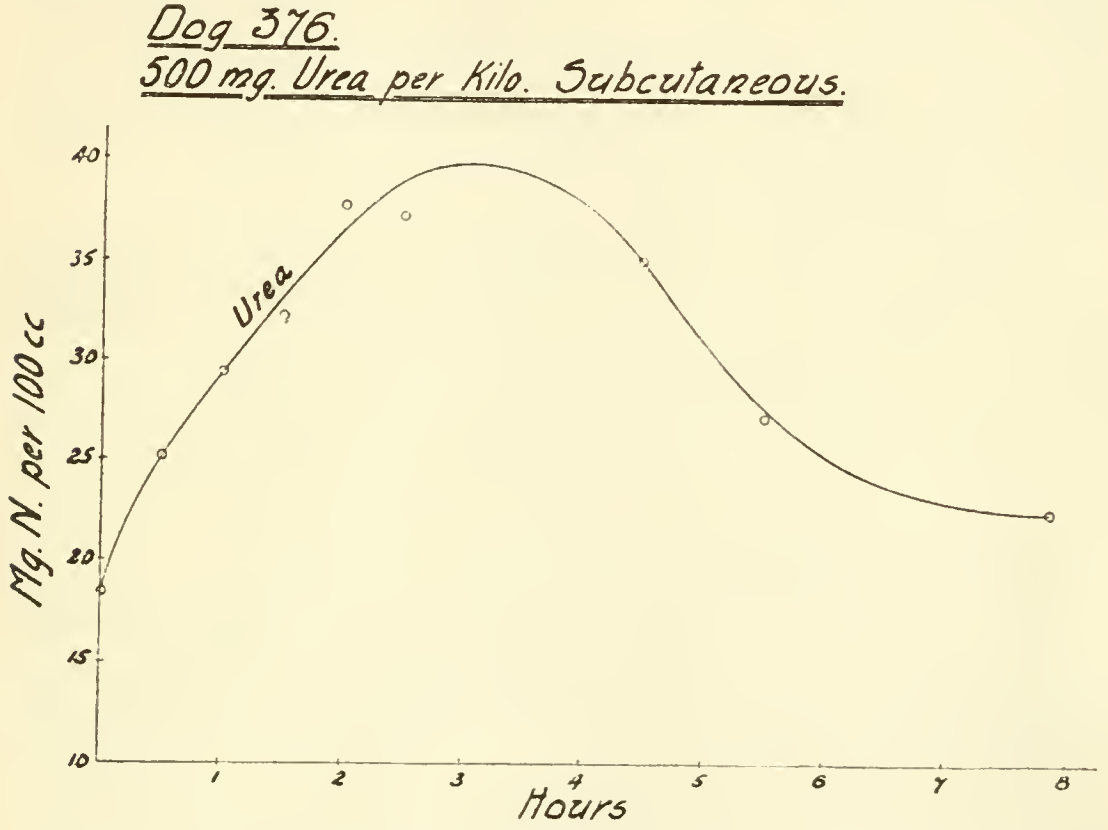

CHART 23. Dog 376, ♀, 8 kilos. Four grams of urea in 20 cc. of water injected subcutaneously. Curve shows subsequent effect upon blood urea.

by intravenous injection if the injection rate is sufficiently slow. Salveson has shown that injected calcium leaves the circulation very rapidly. This has been confirmed but it has also been demonstrated that a blood serum calcium content of approximately 20 mgm. per 100 cc. is borne well even when it is maintained (by artificial means) over many hours. When the blood serum calcium is maintained at this level by repeated injections of calcium chloride the blood phosphorus (inorganic) is increased but not to the extent seen in parathy-

roid hormone overdosage. Our results in this particular are in accord with those of Salveson.

Binger has shown that the injection of either neutral or basic phosphate solutions lower blood calcium and induce tetany. Acid phosphate solutions, however, did not produce tetany but did cause a decrease in blood calcium values. Tisdall (1922) obtained similar results. We have found that phosphate mixtures adjusted to the pH of blood are well tolerated if calcium chloride is also administered in adequate amounts. Under these conditions the blood calcium and blood phosphorus (inorganic) tend to be kept at much lower levels than when acid phosphate and calcium are administered. No doubt in the former case both the injected calcium and phosphate are very rapidly disposed of as Ca_3PO_4.

Not only are the terminal symptoms of parathyroid hormone overdosage simulated by intravenous injections of calcium chloride and acid sodium phosphate but also the post-mortem findings are practically identical. There is marked congestion of the alimentary canal with diffuse hemorrhage through the mucosa into the lumen. Microscopic sections of the wall of the stomach are practically indistinguishable from similar sections obtained from the tissue of an animal killed by hormone overdosage. (Compare figures 2 and 3.)

One may conclude therefore that it is a combination of hypercalcemia and hyperphosphatemia associated with a condition of acidosis which kills. The cause of the preterminal hyperphosphatemia in parathyroid overdosage in dogs and cats is not yet absolutely revealed. Prolonged hypercalcemia in itself, may be the sole cause of the delayed hyperphosphatemia. One cannot state with absolute assurance, however, that this is the only causative factor. It would appear that the unequal response of different species in phosphate mobilization may be an explanation of the variable tolerance to the active extract as seen for example in the dog and the rabbit.

Oral administration of calcium lactate in conjunction with subcutaneous injections of the parathyroid hormone

Calcium therapy as an adjuvant in connection with treatment by means of the parathyroid hormone has been deliberately avoided in all the earlier work with the active extract. This was done in order

that the merits of the hormone might be proven. Now that this has been done, it would seem obvious that a rational therapy should consist of a combination of calcium given by the oral route and of the active extract given by subcutaneous injection. The effectiveness of the hormone is greatly enhanced when calcium lactate is given per os. Animal experimentation has shown that there may be an element of danger in the oral administration of calcium lactate. Thus we have observed most profound hypercalcemia resulting in death in a period of five hours as a result of the oral administration to a fasting dog of 10 grams of calcium lactate at intervals of one hour. In this experiment the drug was administered by the use of the stomach tube and considerable water was used as a vehicle. The blood serum calcium and the whole blood inorganic phosphorus curves obtained in this experiment are shown in chart 15. With the development of the condition of hypercalcemia the blood became very viscous and the final sample was so viscous that only 1 cc. of serum was obtained from 20 cc. by the use of a high power centrifuge. At death the stomach and intestine were hyperemic but hemorrhage had not occurred into the lumen.

This remarkable result is attributed to the filling of the alimentary tract with a solution of a soluble calcium salt. Absorption would thereby be facilitated in the upper and excretion inhibited in the lower part of the intestine. Attempts to duplicate this experiment have so far failed. The usual effect of repeated oral administration of calcium is shown in chart 16.

This latter result stands in marked contrast to those obtained by the feeding of the lactate in solid form along with meat or by the intravenous injection of a solution of calcium chloride and the simultaneous injection of a moderate dose of the hormone. (See charts 12 and 13.)

Dr. Aub[5] has observed that certain human subjects showing only slight response in the blood serum calcium on 50 units of extract per diem developed serum calcium values as high as 19 mgm. per 100 cc. when calcium lactate was added to the diet.

It is therefore evident that, by the intelligent use of a combination

[5] Personal communication. Quoted by courtesy of Dr. Aub.

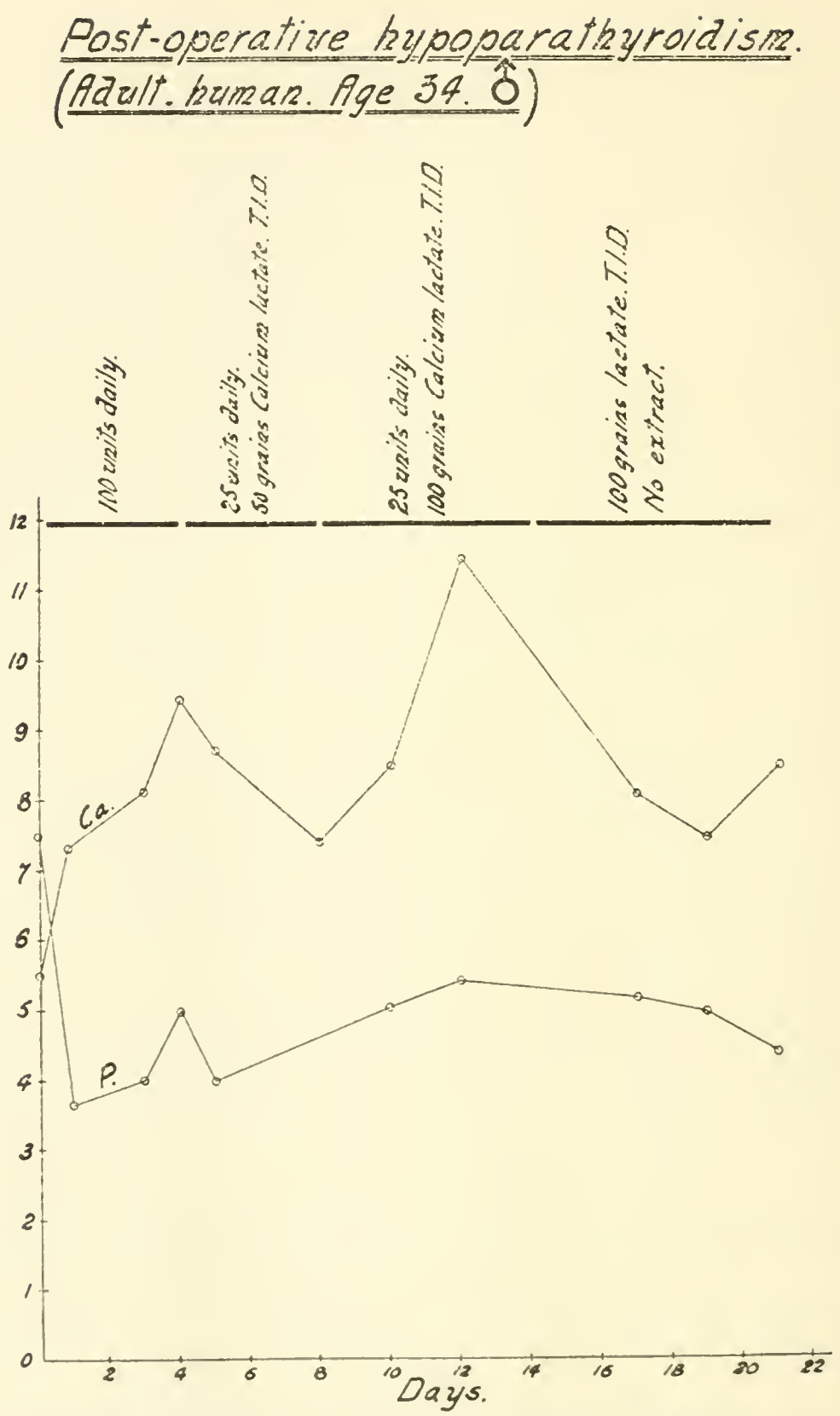

CHART 24. Post-operative hypoparathyroidism treated with parathyroid hormone and calcium lactate as indicated on the chart. An excellent clinical result was obtained

of calcium therapy and injections of the parathyroid hormone, calcium metabolism can be influenced just as surely as sugar metabolism is influenced by insulin.

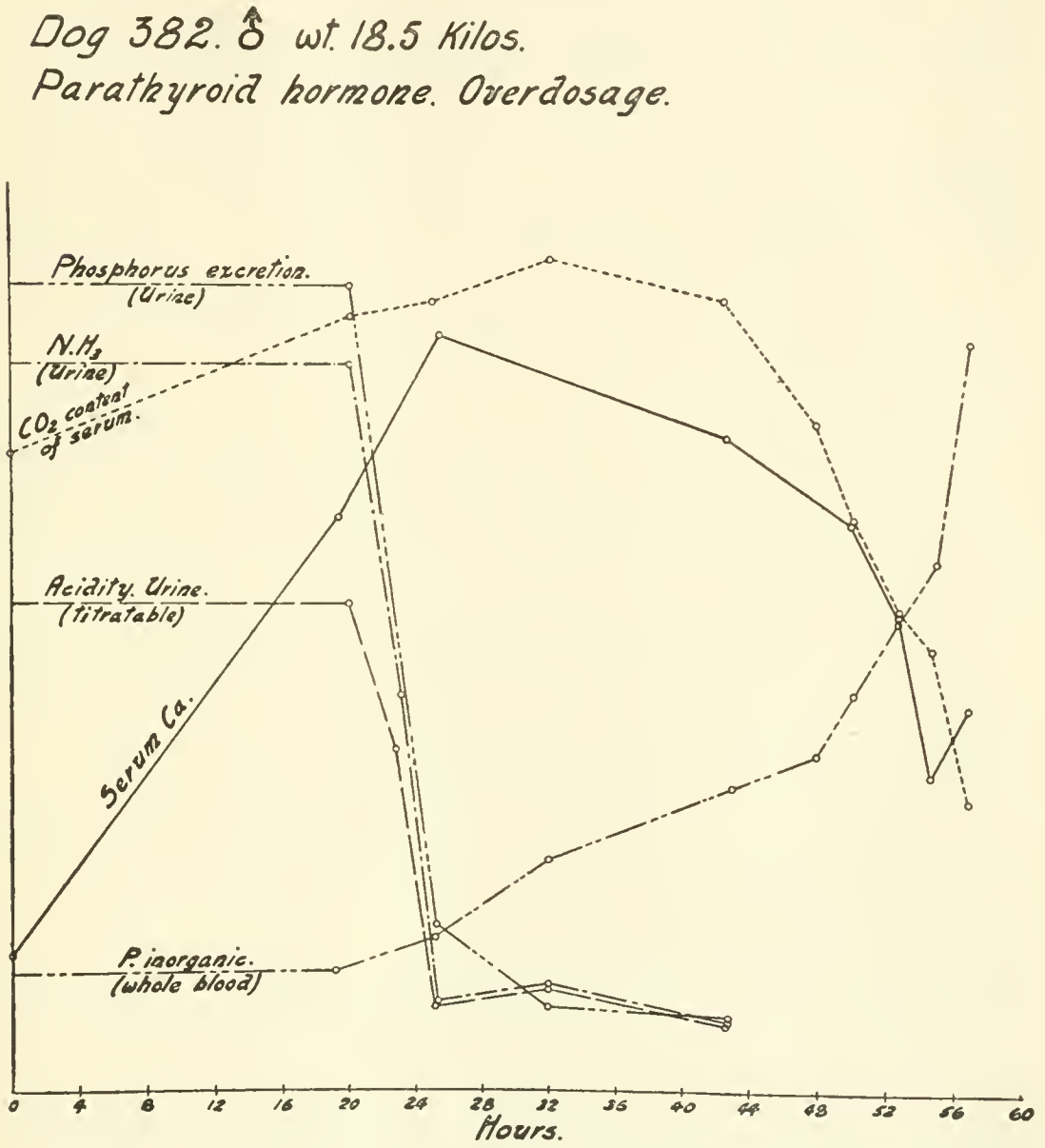
Dog 382. ♂ wt. 18.5 Kilos.
Parathyroid hormone. Overdosage.
Phosphorus excretion. (Urine)
N.H3 (Urine)
CO2 content of serum.
Acidity. Urine. (titratable)
Serum Ca.
P. inorganic. (whole blood)
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
Hours.

CHART 25. Showing the effect of parathyroid hormone overdosage upon the CO_2 content of the serum, the serum calcium and whole blood inorganic phosphorus. The rate of excretion of phosphorus and ammonia in the urine and the titratable acidity are also illustrated. Note that there is almost complete cessation of kidney function in the latter part of the experiment. It is in this period that phosphorus, urea and non-protein nitrogen increase in the blood. The pH of the serum may increase very slightly during the first half of such an experiment. In the last few hours just prior to death the pH is markedly decreased.

The parathyroid hormone as a useful therapeutic agent

Sufficient clinical investigation with the active parathyroid extract has now been carried out to indicate that it will have a very definite place in the therapeutic armamentarium. It will be of greatest value no doubt in those cases in which a condition of hypoparathyroidism exists. Post-operative tetany and infantile tetany are generally recognized as belonging in this class. Recent researches of Scott, Ashford, White and Fleming and others would seem to disclose a condition of hypoparathyroid function in tropical sprue. Further work may show that certain other conditions may be complicated by a mild hypoparathyroidism. The use of the hormone would appear to be definitely indicated as yet only in those cases in which hypoparathyroidism exists.

As calcium enters into the mechanism of so many physiological processes, it is possible that clinical research will later indicate that it is desirable under certain definite circumstances to produce a state of mild hypercalcemia.

A number of cases of hypoparathyroidism in which the hormone has been successfully used have been reported.

Collip and Leitch reported a case of tetany in a young child successfully treated with the parathyroid hormone.

Davidson has reported the use of the active extract in a case of chronic tetany associated with myxedema and nephrosis.

Crile has reported the successful use of the active extract in a number of cases of post-operative tetany.

Snell has reported a case of chronic post-operative tetany treated with calcium and the parathyroid hormone. The dosage of each was so adjusted in this case that neither agent alone would maintain the level of blood calcium within normal limits, while combined, the desired result was obtained.

Lisser has treated a case of post-operative tetany with the hormone with excellent results.

Collip and Blais have recently treated a case of post-operative hypoparathyroidism of unusual type. A most satisfactory result was obtained. The blood chemistry of this case is shown in chart 24.

Petty, Stoner and Schaffer have used the hormone in a number of

very carefully controlled cases and report on the specific effect of the extract in the mobilization of blood serum calcium.

Numerous unpublished clinical reports have been furnished to me through the kindness of many clinicians in various parts of the continent, who have been using the extract in an experimental manner. The results in general corroborate entirely the experimental results obtained in the laboratory.

Experimental clinical work is now being carried on in conditions other than hypocalcemia. As results are obtained they will be published.

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PRESENT KNOWLEDGE OF FILTERABLE VIRUSES

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As all have realized in attempting to review this obscure subject, the first difficulty lies in deciding what diseases are to be considered. Any survey of the progress of our knowledge through the last forty or fifty years will show that during this time we have made enormous strides in emerging from that rather cloudy state of ignorance which prevails when we have succeeded in delimiting diseases merely on the basis of their clinical course even when supported by a knowledge of their pathological anatomy. This progress is due in each instance to the recognition of the aetiological factor and in the case of an infectious disease we must not only see the living cause of the disease but we must learn its zoological or botanical relations and its whole life history. In the course of these years we have learned all this about a host of bacterial and protozoan infections and have come to understand completely the part played by more highly organized animal and plant parasites.

More recently we have realized that there are other groups of organisms which live as parasites and cause disease, but of the systematic position and of the life history of these we are less well informed. Such are the spirochaetes which we cannot yet classify with absolute certainty either with lowly plant or animal forms. While we know a great deal about the life history of some of them, we are still much in the dark about others and it is a little disturbing to find that some are transmitted by ticks or mosquitoes while others are not dependent upon such carriers. It does not seem probable that any essential part of the cycle of their development takes place in these intermediate hosts but rather that they are mechanically transferred much as the plague bacillus is transferred by fleas. At this point it may be said that the part played by such insect carriers seems to be different in different diseases and it is impossible to con-

clude from the knowledge that a certain disease, such as dengue, is transmitted by mosquitoes, that it is caused by a protozoan or a spirochaete. The precise relation of many of these organisms to their intermediate hosts or carriers remains to be worked out.

Another group of organisms which we are only beginning to know is that of the Rickettsias. These minute granule-like bodies occurring often in great masses within cells, often extracellular, are usually parasites or commensals in insects. Their systematic relations are by no means established and little is known of their life history. They do not pass through a filter but are transmitted to man and animals by their insect host, causing such diseases as typhus fever. A third group also of minute granule-like organisms which will only exceptionally pass through a filter and of which we know nothing more as regards the life history, is recognized only because they cause such diseases as smallpox.

After this it becomes exceedingly difficult to group together on any rational basis the aetiological agents of the many remaining infectious diseases of which we have a still more unsatisfactory knowledge.

Most of these diseases seem to be caused by some living creature so small as to pass readily through a porcelain filter which will hold back bacteria and other relatively bulky organisms. They must be very small but it is impossible to think of filterability through a Berkefeld or Chamberland filter as a satisfactory basis for classification so that the title of this paper becomes misleading. For no two filters are equally permeable, and many external circumstances modify their permeability. Pressure, the degree of dilution of the fluid to be filtered, its viscosity, the presence of viscous sediments which may clog the filter, the possible existence of various stages in the development of the organism with corresponding differences in size and consistency,—all these things affect the passage of organisms. Indeed, Dr. Holman has shown that the slightest smearing of a filter with oil will make it permeable. All this is of great interest in the case of such organisms as pass with difficulty through filters—for example, those of smallpox and vaccinia which seem to have been driven through filters by some investigators although it was evident that but few of them went through and only when the material was greatly diluted and freed of viscous impurities..

On the other hand there are many diseases the living virus of which passes readily and in full strength through every kind of porcelain filter. Of these there are some in which it is easy to demonstrate this fact because the disease can readily be reproduced by the inoculation of the clear filtrate but there are others, severe or fatal diseases of human beings, which have every appearance of being of the same nature in which filterability of the virus has not been demonstrated probably because no other animals are susceptible and no one has ventured to inoculate human beings. Rheumatism is apparently such a disease.

The list of these diseases is a rather long one and one group seems to be set apart because of the predominant involvement of the central nervous system in which there are produced profound destructive changes. This group includes poliomyelitis, encephalitis lethargica, herpes, rabies, distemper of dogs and probably other diseases such as Borna's disease of horses. But in spite of the similarity of the pathological changes, it is by no means certain that the causative agents are intimately related.

We have no good basis for grouping such diseases as equine distemper, African horse sickness, fowl pest, foot and mouth disease, hog-cholera, rinderpest, influenza, mosaic disease of tobacco and other plants, infectious anaemia of horses and infectious leukaemia of fowls, nor do we know where to class the pleuropneumonia of cows and of horses, measles, scarlatina, dengue, rheumatism, verruga, mumps, molluscum contagiosum, warts and trachoma. There are ten or twelve other diseases of local occurrence which are equally difficult to classify although it is known that they are produced by a filterable virus.

In many of the diseases mentioned there are curious inclusions in the affected cells either in the cytoplasm as in smallpox, vaccinia and the related infections and in rabies and trachoma, or in the nucleus as in herpes. Much attention has been concentrated upon these but although their presence is recognized as constant and specific for each disease so that they have a great diagnostic importance, there is no real knowledge of their nature. While some hold them to be a part of the life cycle of the parasite, others maintain as stoutly that they are merely the result of degenerative changes in the cytoplasm or nucleoplasm of the cells.

Morphological studies after all have been of relatively little value in the attempt to unravel the nature of these peculiar diseases. Cultivation of the parasites has almost always failed—the viruses of pleuropneumonia of cattle, poliomyelitis and vaccinia are almost the only exception and in those it has succeeded only after the most extraordinary efforts. Separation of the virus by filtration and the transmission of the disease by inoculation is the main procedure in these investigations and this depends upon the availability of susceptible animals. Of course when the same species can be used it is a simple matter but when the virus must be inoculated into some other animal difficulties are encountered not only because of the new medium in which the character of the virus may suffer a profound change, but because of the confusion often produced by the spontaneous occurrence in the test animal of some other virus, independent, but similar to that inoculated.

The testing of the immune reactions produced in these diseases has frequently thrown light on the specific relation of the virus to the disease although this is usually limited to the observation of the protection of actively and passively immunized animals against the inoculation, and of anaphylactic reactions in immune animals.

Of the whole list, if we except the Rickettsia infections and the smallpox group in which the recognizable infective agents are still on a rather precarious basis, there is not one in which we have satisfying knowledge of the organism and its life cycle. Poliomyelitis is doubtless best known of all—from the work of Flexner, Lewis, Noguchi, Amoss and others it is known that this is a virus which can be inoculated into monkeys, producing the disease and can be isolated in a water-clear filtrate in which nothing can be seen but which is still potent to produce the disease, and can even be cultivated in visible form upon media containing fresh tissue.

Pleuropneumonia of cattle seems to be caused by a minute organism named by Nocard and Roux, Asterococcus mycoides, which can be cultivated in various media and will reproduce the disease. But after this in a list of perhaps forty diseases nothing is known of the nature of the organism except that it will go through a filter. It seems a poor record after all these years of effort and in spite of the outpouring of an oppressive amount of literature, that so very little

should be known with certainty. Progress has been made in the study of Rickettsia infections, of the poliomyelitis-encephalitis-herpes group and to a less extent of the smallpox-vaccinia group, and these we may discuss with some mention of the recent studies of scarlet fever, measles, influenza, rheumatism, etc., among the human diseases.

Yellow fever is almost universally regarded as settled in the light of Noguchi's discoveries, but one is still curious about the difficulty in finding the leptospira, its intimate relation with the leptospira icterohaemorrhagica which is so often present in rats in the places where yellow fever is found, and especially about the obligate relation of the infection to one species of mosquito and the period of twelve days which must elapse before the mosquito which has bitten a yellow fever patient can infect another person. All of these things seem far more compatible with the idea that yellow fever may be caused by some organism specifically related to the Stegomyia and like malaria completing part of its life cycle in the mosquito.

RICKETTSIA INFECTIONS

It seems fairly clear from the work of Ricketts, da Rocha Lima, Prowazek, Jungman, Wolbach, Palfrey and Todd, Weigl, Kuczynski, Cowdry, Sellards, Nagayo and others, to mention only the most familiar names, that these peculiar organisms are directly concerned in the production of several diseases. Rickettsia prowazeki, transmitted by lice, seems to be clearly shown to be the cause of typhus fever; it is an intracellular parasite in the intestinal epithelium of the lice which have fed on a person ill of typhus. How it is transmitted to the next person is not clear except that such lice by biting a normal person can transmit the disease whether by the actual bite or by the infection of this puncture wound with their faeces. The organism is found in the endothelial cells of capillaries in persons dying of typhus, it is not filterable and has been cultivated only in growing tissue in vitro by Wolbach and in these cultures it survives and multiplies only so long as the tissue remains alive. Immunological relations, such as agglutinin formations, are rather specific and protection is obtained in guinea pigs inoculated with Rickettsia prowazeki against inoculation of typhus blood.

Trench fever, or Wolhynic fever, is apparently caused by another Rickettsia which is extracellular in the louse intestine. Strong found a filterable virus in cases of trench fever which is opposed to this, but there is much evidence (Bacot, Jungman, Töpfer and others) that the presence of Rickettsia quintana, or Wolhynica or pediculi in the body of the lice is a specific result of feeding on trench fever patients and that their infectivity depends on this.

Rocky Mountain spotted fever is clearly shown to be due to an allied form, Dermacentroxenus rickettsi which is transmitted by a louse, Dermacentor venustus. The organism is not filterable but has been cultivated or at least maintained in tissue cultures of testes in vitro (Wolbach and Schlesinger).

Cowdry has shown that the heart-water disease in sheep is definitely caused by Rickettsia ruminantium which is transmitted by Amblyomma hebraeum (a tick), and quite recently Sellards, Nagayo and others have found a Rickettsia in Tsutsugamushi disease which is pathogenic for guinea pigs and monkeys and transmitted by the mite Trombicula akamushi.

On the whole it appears that we may regard this as a group of infections, not, it is true, caused by a filterable virus but difficult of study and obscure, and therefore mentioned here because this is practically the definition of our subject rather than filterability. It is good that such satisfactory progress has been made in clearing up these diseases and with these examples before us there seems little doubt that the further exploration of the relations of this type of organism which seems to be widely represented in insects at least, may be fruitful and relatively easy.

THE SMALLPOX-VACCINIA GROUP

Although, of course, these diseases have been practically well known for ages and are readily transferable and have furnished us with our first classical idea of artificial immunity, the nature and life history of the organisms are but little understood. That the organisms are very minute is well known; that they are really readily filterable is very doubtful. Some authors have succeeded in driving vaccine through filters—others have failed, and in the most successful attempts the quantity of the virulent material has been greatly reduced. The

organism of vaccinia has been cultivated in living tissue cultures in vitro but not otherwise. Its increase has been shown by the more profuse effects upon animals (Steinhardt and Lambert), or by the calculation of dilution and time of maintenance. It is evident that if a tissue culture inoculated with a minimal amount of vaccine, perhaps even less than is necessary to infect a rabbit's cornea, is transferred many times throughout a long period in the incubator which would in itself be enough to kill the virus, and is then found abundantly able to infect the rabbit's cornea, the virus must have increased greatly (Parker and others). Prowazek, Aragao and Beaurepaire, Paschen, Gins and many others have recognized minute granules in vaccine and all have observed the inclusions (Guarnieri bodies) in the epithelial cells. MacCallum and Oppenheimer have separated the granules from the rest of the vaccine by differential centrifugalization and in work now going on Oppenheimer has washed these granules free of all adherent material and found them infective and Craciun and Oppenheimer have been able to cultivate the washed granules in tissue cultures for many generations.

Doubtless all this will hold true for the closely allied affections—the milder forms of variola, alastrim, etc., horsepox, goatpox, swinepox, sheeppox, which seems of all these the most independent disease, and finally the fowlpox or epithelioma contagiosum of fowls. But it leaves us ignorant of the systematic position of these minute granules and of most of their life history, even of their relation to the cellular inclusions which are so characteristic. Nevertheless, the progress of late years is very definite and promises rapid advances in the near future. Varicella, or chickenpox, has always been ranged with these diseases and it is quite possible that it belongs here although as will be related, attempts have recently been made to align it with another group—that of herpes.

POLIOMYELITIS, ENCEPHALITIS, HERPES GROUP

The most intense interest has been concentrated upon these diseases recently because of their wide and fatal epidemic occurrence. The study of poliomyelitis is now set aside as practically completed and little new work has appeared in recent years. The main points have already been referred to and it seems that with practically com-

plete knowledge of the clinical symptoms and the anatomical changes, and with the demonstration of a filterable virus capable of reproducing identical conditions in monkeys and of being cultivated in artificial media, the comprehension of the disease should be satisfactory. But we cannot see the organism ordinarily, except in the form of globoid bodies in incubated tissues and in cultures and since we know little of the relation between these granules and the invisible virus in the water clear filtrate which is yet so potent, it can hardly be said that we understand the life history of this organism. But then we might say that it is possible that we are equally in the dark about the whole life history of the most familiar pathogenic bacteria.

With regard to encephalitis lethargica we now know well the clinical course and the pathological anatomy but we are entirely ignorant of the nature of the causative agent. It is true that various authors have announced the discovery of a virus which can be transmitted to animals such as rabbits, but the evidence is unsatisfactory. Strauss, Hirschfeld and Loewe transmitted the disease to rabbits very readily and were supported by Thalhimer. Levaditi and his coworkers also succeeded in producing encephalitis in one rabbit by inoculating material from the brain of a person dead of encephalitis and in another by inoculating nasal washings, and all their subsequent work has been with these two viruses. Doerr and his associates have secured another virus in the same way. But all of these are open to criticism chiefly on account of the discovery by Grüter, Loewenstein and others that the virus of herpes simplex is very readily inoculated into rabbits and produces in them a fatal encephalitis. This has been confirmed and studied by so many investigators that it is perfectly established and Lipschütz has described a specific intranuclear inclusion which is characteristic of herpes lesions. There is no difficulty in transmitting herpes to rabbits, but enormous difficulty in transmitting encephalitis lethargica. Some writers, such as Bastai and Busacca, even find the herpes virus in the blood and cerebrospinal fluid of persons who are subject to herpes but are at the time in an interval without any actual herpes, and say that in such people any slight disturbance is capable of producing a local herpetic skin lesion. This is denied by Lipschütz but it is at least enough to suggest that persons with encephalitis might sometimes harbor the virus of herpes which

would account for the rare examples of successful inoculation of rabbits.

The course of herpes simplex infection has been minutely and satisfactorily studied by Goodpasture and Teague who show that the virus wanders rapidly along the nerves, choosing as its path the axis cylinders and kept in place by the myelin sheaths, spreads out only upon reaching a ganglion or the central nervous system. Short of a knowledge of the morphology and life cycle of the herpes virus, this seems fairly satisfactory but the easy recovery of this virus from occasional non-encephalitic cases such as that of Flexner and Amoss, and the extraordinary difference between the curves showing the effect of inoculation of encephalitis material on the one hand and of herpes material on the other, into rabbits (Ford and Amoss), make one suspect very strongly that all of the so-called viruses of encephalitis are really accidentally recovered herpes virus. This seems a far safer conclusion than that ventured by some that the viruses of encephalitis and herpes are identical, and it is preferable to concede that as yet we know nothing of the cause of encephalitis. All of this has been clearly brought out by Flexner.

In the course of all this groping toward the herpes virus and that of encephalitis, great confusion has been introduced by finding in the rabbit, the common experimental animal in every laboratory, a spontaneous infection which causes a sort of encephalitis. Many have now written descriptions of this organism which is very common in rabbits and which has been classed as a form of microsporidium. Except that it must be recognized to avoid confusion, it has no further interest here. Levaditi has named it Encephalitozoon cuniculi.

Nearly related to herpes simplex in our minds is herpes zoster, and beginning with the paper of Bokay there has sprung up the idea that this too may be a transmissible disease produced by a filterable virus and also affecting the nerve and related ganglion. Such an idea is in every way acceptable although combated by many clinicians who feel sure that herpes zoster arises as a secondary effect after many diseases (Comby). It is extremely difficult to transmit herpes zoster to animals although a few, such as Lipschütz, Marinesco and Draganesco, report positive results. Luger and Lauda thought they produced herpes simplex encephalitis by inoculating herpes zoster material and

Cole and Kuttner in their recent studies were quite unable to transfer herpes zoster at all. At least it seems immeasurably more difficult to transmit to animals the virus of herpes zoster than that of herpes simplex and it is doubtful that it has ever been done—once more because of the chance of the accidental presence of herpes simplex virus which might give an illusory success. But the most interesting thing is that Bokay thought that inoculation of herpes zoster might be followed by the development of varicella or chickenpox and vice versa. This has been discussed by a great many, especially in France where Netter and Urbain stoutly maintain that varicella and herpes zoster are only different expressions of the same infection. Clinicians such as Comby are equally forceful in maintaining the complete independence of the two diseases. Lipschütz and Kundratitz have, they say, transmitted herpes zoster to children in whom it often spreads into a general vesicular eruption like chickenpox, easily transmitted to other children by contact as chickenpox. Children who have had varicella are refractory to the inoculation, and those who have been successfully inoculated are immune from chickenpox. Even the serum of convalescents from herpes zoster is said to be protective against varicella. But Lauda and Silberstern deny this and fail to confirm the cross complement deviations of Netter with the serum of zoster patients and varicella antigen.

From all this it is difficult to draw any certain conclusions. It seems quite clear that herpes simplex is caused by a definite filterable virus transferable to rabbits in which it produces fatal encephalitis, and there are suggestive fragments of evidence that it is very widespread and may even exist coincidentally with other infections, and thus lead to confusion when susceptible animals are inoculated from encephalitis or other diseases. But it is by no means easy to feel convinced by any of the experimental studies which appear to demonstrate the causative agent of encephalitis lethargica. Whether herpes zoster is a separate disease caused by a virus distinct from that of herpes simplex is quite uncertain but it seems to be so because it is so difficult to transmit it to rabbits which are so susceptible to infection with herpes simplex. That it has any special relation to chickenpox rests almost entirely on circumstantial evidence and seems at least improbable. But none of this complex field is ripe for discussion and we must await the results of much more experimental study.

In this group there belong perhaps the enzoötic encephalitis of horses (Borna's disease), described by Moussu and Marchand as caused by a filterable virus infective for the rabbit and producing in that animal as well as in the horse, a fatal encephalitis. Whether distemper in dogs with its well recognized lesions in the central nervous system is due to such a virus or really to the filterable bacillus bronchisepticus remains an open question (Roman and Lapp).

It may perhaps seem strange and improper to group rabies with this class of diseases too, but in its general behavior it seems to me closely related. Nothing new has been discovered about rabies unless it be the suggestion of Manouelian and Viala that the Negri bodies represent a stage in the cycle of a microsporidian analogous to that described by Levaditi as Encephalitozoon cuniculi.

OTHER DISEASES CAUSED BY FILTERABLE VIRUSES

Of all the other diseases caused by filterable viruses we are almost entirely in the dark, and it will be tedious to mention them all merely to repeat this statement. However, there is a little light appearing with regard to some of them and at least the promise of better understanding in other cases.

Thus, in the case of common colds, it seems satisfactorily shown by the work of Kruse, Foster, Olitzky and McCartney that bacteria can be excluded by filtration and that a cold may be transmitted by the clear filtrate of nasal secretions from a person in the early stage of a coryza which has been contracted by contact. But that is all and it is only because this is a common disease which affects us all that this information is any more interesting than the similar information which is all we possess concerning certain diseases of horses, such as aphthous stomatitis, equine distemper, pleuropneumonia, African horse sickness, and infectious anaemia, or certain diseases of cattle such as foot and mouth disease, rinderpest, stomatitis papulosa and agalaktia contagiosa. Other similar diseases affect swine—hog-cholera, or sheep, catarrhal fever, Nairobi sheep disease, or fowls, fowlpest, infectious leukaemia of hens, etc. There are other diseases still, affecting rats and guinea pigs, rabbits, birds, silkworms, etc., and doubtless a host of others which we have never noticed. Great excitement was aroused by the statements of Frösch and

Dahmen that they had discovered the cause of foot and mouth disease by photography by ultraviolet light, but this is left to lapse into silence by the commission of the German Microbiological Association appointed to investigate it and similar skepticism seems to prevail with regard to the analogous discovery by Barnard of the cancer parasite by the same method. But there still remain several human diseases of great importance which have not exactly fallen into any of these classes although they seem to be closely related usually because they behave like those which are due to a filterable virus. Such are influenza, measles, scarlatina, dengue, pappataci fever, rheumatism, verruga and after these the local affections, molluscum contagiosum, warts and trachoma.

As to *influenza*, the efforts of almost all the workers in the world failed to discover the etiological factor when the most gigantic material was offered but at least the impression became general that the bacillus of Pfeiffer could claim nothing more than the rôle of a secondary invader and that there must be a filterable virus although no one could demonstrate it. The work of Olitzky and Gates on the Bacillus pneumosintes, a filterable bacterium, is extremely interesting but does not convince one at once that in that organism the cause of the epidemic is found. No one has actually reproduced the disease in characteristic form in spite of many efforts and it remains an unsolved problem.

Measles studied by the usual methods has shown itself to be a disease caused by a specific virus which is found in the blood, nasal and conjunctival secretions, and elsewhere in the body. It will pass through a porcelain filter and will reproduce the disease when injected into susceptible human beings or monkeys. Hektoen demonstrated this clearly and his results were confirmed by Goldberger and Anderson and later by Blake and Trask, although Sellards had arrived at an opposite conclusion. Others have described similar infection of rabbits and guinea pigs (Nevin and Betman, Duval and D'Aunoy), but whether these are convincing or not there seems little doubt about the fact that man and monkeys can be thus infected. None of these experiments resulted in any demonstration of a visible organism although Mallory described minute bodies in the endothelial cells of capillaries in the exanthem. Recently Caronia and Sindoni

have found in the blood, bone-marrow, filtrate from nasal secretions and cerebrospinal fluid of infants in the prodromal or exanthematic stage of measles, an organism which grows anaerobically in catalytic media and which is visible as very minute granules in pairs. This organism will pass through a porcelain filter and from the clear filtrate new cultures may be made. Inoculation of large doses of the culture intravenously produces a disease like measles in rabbits and the organisms may be recovered. The serum of children convalescent from measles specifically agglutinates these organisms. Inoculation of inactivated or attenuated cultures produces immunity from measles in children while large doses of the living culture produce typical measles. de Villa has shown that inactivated cultures injected into the skin produce a reaction like the Schick reaction in non-immunes but not in immune persons. Various others have found the organism (Arloing and Dufourt, Ritosso, etc.), and Dr. Saphro is now working with it in our laboratory. She has succeeded in cultivating it from cases of measles and although we have seen that it corresponds in its morphological and cultural characters with the cultures brought from Caronia's laboratory, we must await further study.

Dengue, which resembles measles in some respects, has been shown by Graham, Ashburn and Craig, Cleland, Bradley and McDonald and others to be transmissible to human beings by inoculation of blood even when filtered through a porcelain filter. The organism is thought by Chandler and Rice not to be a spirochaete although some authors have suggested this. Graham thought it an amoeboid body within the red corpuscles but no one has confirmed this. Duval and Harris think they have transmitted the disease to guinea pigs as shown by fever, fall in leucocytes and enlargement of the spleen and have cultivated certain globoid bodies for many generations which will produce the same results when inoculated into guinea pigs. Great interest centres in the relations with mosquitoes which naturally transmit the disease. While Graham and Ashburn and Craig thought that Culex fatigans or quinquefasciatus was the natural carrier, Bancroft, Cleland, Bradley and McDonald, Chandler and Rice, and most recently Siler, Hall and Hitchens have shown that the Stegomyia fasciata (Aedes aegypti) is really the vector and not Culex fatigans. The latter group of authors show that mosquitoes can

receive the virus from patients in the first, second and third day of the disease, that the virus must remain in the mosquito eleven days before transmission is possible. Immunity follows the disease but is not absolute and most persons who live in countries where dengue is common go through several attacks in successive years, the illness becoming milder each time until they seem to be quite immune. From this we may conclude that the organism has yet to be discovered but that like that of malaria it seems to require time to complete some part of its life cycle in the mosquito. This, of course, is not a strict consequence but otherwise it is difficult to explain the eleven day period unless we merely assume that the organism grows in the mosquito until the dosage becomes sufficient to produce infection. It would be interesting to determine whether one mosquito after eleven days could infect several persons.

Of *scarlet fever* there is little to say here. De Cristina, Caronia and his associates, and others have found an organism like that described in measles but others who have attempted to repeat this work have failed to confirm it (Burgers and Bachmann, Meyer). It has, of course, always been traditionally held that scarlet fever is an exanthematic disease closely related to such diseases as measles and leaving behind it a lasting immunity, but generally complicated by a secondary infection with streptococci. The immunity and various anatomical changes seemed characteristic of scarlet fever in contrast with what is found in uncomplicated cases of streptococcal infection. Now it appears from the work of Dochez and of Dick and Dick that a special streptococcus is concerned, that this as Dochez says is constantly associated with the primary and secondary manifestations of the disease, it is specific in character, able to produce the experimental disease in man and animals, its toxic effects are neutralized by the serum of human convalescents. On the other hand the specific toxic phenomena of scarlet fever in man can be made to disappear by an antitoxic serum produced in the horse by this streptococcus, and a Berkefeld filtrate from cultures of the organism is found to contain a toxin which is specifically related to immunity in scarlet fever as shown by skin tests. From all this Dochez concludes that the Streptococcus scarlatinae is the principal and probably the only aetiological agent of scarlet fever.

Pappataci fever or phlebotomus or three-day fever, which is so widely distributed in the tropical and Mediterranean countries, was shown by Doerr and Russ and Birt to be due to some filterable virus which circulates in the blood and which upon injection into susceptible persons will produce the disease. It is transmitted by a sort of mosquito, the pappataci (Phlebotomus papatasii) which is able to infect another person only after the virus has been in its body for seven days, which again suggests that some part of the life cycle of the organism must take place in the vector's body. Nothing satisfactory has been done in the way of recognizing precisely this organism. Whittingham thinks it may be a leptospira but it is not pathogenic for guinea pigs and not inherited by the phlebotomus.

Rheumatism is quite clearly an acute and recurring specific infectious disease producing absolutely peculiar and characteristic anatomical changes. No bacteria have been satisfactorily recognized as the causative agent and in spite of many attempts no one has succeeded in producing the disease experimentally in any animal. As far as recorded no one has attempted to infect a human being with this extremely serious disease, and although it seems possible that it may be due to a filterable virus, there is no evidence upon which to base any statement.

Verruga peruviana. No perfectly clear idea of the aetiology of this disease is yet available in spite of the considerable amount of attention recently paid it. The general opinion which prevails among the investigators in Peru is that Oroya fever in which peculiar minute bacillus-like organisms are found in the red blood corpuscles (Bartonella) is the first stage in a disease in which later the nodules or verrugae appear on the skin and elsewhere. Strong and his associates, however, believe that the Oroya fever and verruga are separate diseases. They could not transmit Oroya fever to animals but could inoculate monkeys and other animals with verruga although they could not demonstrate a filterable virus nor any definitely visible organism.

As to common *warts*, Jadassohn was able to transmit them by inoculation. Ciuffo could filter the material and still produce them by inoculation and Wile and Kingery have not only reproduced them in human beings by filtrates but have transmitted them in the same way to a second generation. In all cases the period of incubation from the time of inoculation to the development of the warts is very long.

Molluscum contagiosum is another disease of human beings which has been reproduced in typical form by the injection of a bacteria free filtrate probably by Juliusberg, certainly by Wile and Kingery. The cellular inclusions are typical but the organism is evidently an extremely minute form. The relation of this to the epithelioma contagiosum of fowls is not settled. That disease which sometimes appears as a diphtheritic inflammation has been mentioned already in relation with vaccinia. It has been shown by Marx and Sticker to be transmissible by the injection of a filtrate and in the emulsion there are found minute granules somewhat like those found in vaccinia.

Nothing new seems to have been accomplished in relation with the various other less-known diseases due to filterable viruses except that Olitzky in working with the virus of mosaic disease of tobacco has been able to cultivate it in a medium made from the plant and to show by calculating the dilution that the invisible virus must have multiplied greatly.

One is left with the feeling that in comparison with the amount of work, and especially with the bulk of the publications in this field, very little has been accomplished. The question arises as to what we really want to know about these viruses and it seems that this may be answered from two points of view. As a matter of intellectual interest we want to know their morphology, their behavior with regard to various environmental conditions, and particularly their whole life history. If we could know the life cycle of any organism, even including the common bacteria, with such precision as we know that of the Schistosomes or of malaria, we would feel content. As a matter of practical interest merely to enable us to combat the disease we might be quite content to know only that this is a filterable virus and that somewhere in its life history there is a point at which its transmission can be attacked as is the case with yellow fever. Even if we doubt the part played by the Leptospira, we know that the virus must be carried by the Stegomyia and it is only necessary to keep this pest from biting the persons who have the disease to exterminate the disease. From this practical point of view immunological relations assume an importance far out of proportion to their interest from the other standpoint, and practically we can control the spread of many of these diseases or prevent their occurrence in an individual.

One has the impression that progress is slow because we still use blindly the methods of investigation which have been worked out with such effort for bacteria. When someone succeeds in devising new conceptions—as new as the use of aniline dyes and solid media were for the early days of bacteriology, progress may be rapid. It seems far from impossible that totally different media may be necessary for the cultivation of these minute organisms and that quite different principles of immunity may prevail. One organism properly worked out should serve as a model for all the rest. It is intriguing to reflect that while we know of the existence of only those filterable viruses which produce disease, there are doubtless thousands of such minute living creatures round about us which will forever escape our perception.

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THE PRESENT STATUS OF OUR KNOWLEDGE OF THE ETIOLOGY OF PELLAGRA[1,2]

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On March 26, 1735, nearly two hundred years ago, a physician, Doctor Gaspar Casal, practicing in the little town of Oviedo, Spain, made some notes on the first of a series of cases of a disease known to him and to the people of the locality as *Mal de la Rosa* (1). That disease we now know as pellagra.

Casal described the symptoms, termination and sequelae and discussed the cause, nature and geographic distribution of the disease; but it is Casal's ideas as to the cause which alone interest us at this time. He thought that the cause resided in a combination of atmospheric conditions and food; food rendering the body susceptible and the atmosphere through an inherent and "malignant" quality acting as the efficient or precipitating cause of the malady. The idea, thus for the first time expressed, that food was etiologically related to pellagra persisted but underwent in the course of time various modifications until the theory was evolved that pellagra is an intoxication resulting from the consumption of spoiled maize. With this theory of the etiology of pellagra the names of Sette (2), Balardini (2), Roussell (3), and more particularly that of Lombroso (4) are closely associated.

Needless to say, there had not been lacking among the older students some who believed that the disease was a communicable one. Such belief, however, had at no time been widely held and it had long been fairly completely dismissed from serious consideration in favor of what had become the authoritative and, in certain countries, the official spoiled-maize theory of Lombroso when Sambon (5), first in

[1] Delamar Lecture delivered before the School of Hygiene, Johns Hopkins University, Baltimore, Md., March 1, 1926.

[2] Published with the permission of the Surgeon-General.

1905 and then more vigorously in 1910, attacked this theory and in its place advanced the view that pellagra was an insect-borne infection probably transmitted by some species of *Simulium* or buffalo gnat.

Thus upon the recognition of the disease as endemic in the United States, American students found these two conflicting views as the outstanding etiological theories and proceeded at once to subject them to critical examination with the result that both were soon rejected. In their place two other outstanding, likewise opposing, views developed: One, that the disease is an excreta-borne infection in which diet may play a more or less important but not an essential rôle; and the other, that the disease is primarily of dietary origin, a diet faulty or deficient in some, at that time, unknown respect being the essential factor in its causation.

The studies of the past ten or twelve years have tended, on the one hand, increasingly to invalidate the conception of pellagra as an infection and, on the other, to establish a more and more secure foundation for the theory of dietary deficiency. Indeed, it may be stated that our knowledge of the nature of the dietary deficiency has now become sufficiently clarified to warrant placing pellagra in a category closely related to, if not identical with, that of beriberi and scurvy. This, it is hoped, will be made clear in the discussion that follows.

It will be helpful to begin this discussion with a brief review of the more important reasons for considering pellagra as primarily of dietary origin.

The first of these reasons is the fact, as will presently appear, that the epidemiological and clinical evidence that has been advanced in support of an infective etiology either has not the significance that has been attributed to it or is equally consistent with the dietary view. Thus we find that the association of the disease with bad hygienic and sanitary conditions has been regarded by many as highly significant of an infection. But such association, it may be pointed out, is just about what might be expected of a disease of poverty and endemic pellagra, it may be recalled, is associated with the condition of poverty to an extraordinary degree (6). Furthermore, the reported studies that seemed to indicate a significant correlation between insanitary conditions, particularly as relates to methods of excreta disposal (7) (8) (9), do not bear critical analysis nor have they been

supported by the results of more extensive studies carried out by later workers (10). If the association with poor sanitary conditions were really significant it would be reasonable to expect that the prevalence of the disease might be reduced or that the disease might even be eradicated by improving sanitary conditions. An experiment to test this was actually carried out by Siler, Garrison and MacNeal (11) in a cotton mill village of South Carolina and, as reported by them, with notable success in eradicating the disease. There is good reason to believe, however, that these workers fell into error in interpreting the result of the experiment for the prevalence of the disease in the experimental village was later found by workers of the Public Health Service to be quite as high as in some other nearby villages with but the crudest methods of excreta disposal (12). It may be stated in this connection, that while there is no sound evidence that the disease can be prevented by improving sanitary conditions, other evidence of the failure of improved sanitary conditions to control the prevalence of the disease has been reported (13) (14).

Some of those who have regarded the association of the disease with poor sanitary conditions as significant, have reported evidence of the occurrence of contact with a preexisting case in a very high percentage of incident cases (7) (8) (9) (15) and have considered some of this as well nigh conclusive evidence of infection. It would be very instructive to examine this evidence in detail but it must suffice to state, as Vedder (17) and Goldberger and Wheeler (16) have pointed out, that much of it is consistent with the conception of a dietary deficiency and that which is not, is the result of faulty or inadequate methods of investigation and really without significance. That "contact" has not the significance that some observers have attributed to it is strongly indicated by its failure to operate under what would seem favorable conditions, as we shall endeavor to make clear presently in another connection.

The marked seasonal incidence of pellagra, its seemingly sudden appearance and alleged rapid extension in the United States with its notably higher prevalence in the Southern States represent epidemiological characters that one naturally associates with infectious diseases, so that it is not surprising that these have been cited in support of an infective etiology. In doing so, however, it was overlooked

that such characters are not peculiar to infectious diseases. Excellent analogies may be found in the literature of beriberi (18) (19) so that they are, manifestly, equally consistent with a dietary etiology.

The notably higher incidence of the disease in adult women than in men has been cited in support of infection on the ground, presumably, that such difference is inconsistent with a dietary etiology. This is, however, by no means necessarily the case as witness, for example, the great preponderance of endemic goitre in girls as compared with boys of the same age (20). While it must be acknowledged that in the light of present knowledge the peculiarities not only of sex but also of age incidence exhibited by pellagra are not altogether satisfactorily explainable on a dietary basis, they are even less satisfactorily explained on the basis of infection. For to suggest a difference in "resistance," virtually the only explanation that the infectionist can advance, is an explanation that is manifestly equally consistent with the dietary theory and it is itself in need of explanation.

Although a satisfactory explanation on a dietary basis of the phenomenon under consideration can not yet be formulated, a number of factors may, nevertheless, be suggested as possibly contributing to bring it about. Thus it is conceivable that during adult life there may be a quantitative difference between the sexes in physiological requirement of the special dietary factor related to pellagra or there may be a difference in the length of the developmental period of the disease so that what, if not interfered with, would be the longer period of evolution of the disease in the male may be conceived as cut short by seasonal changes in diet which do not come quite soon enough however to benefit the female in the same way. It may be suggested further that the housewife may, and on the basis of every day observation doubtless does in some instances, at least, favor the husband and other wage earning members of the household in matters of diet and, also, that the husband and other wage earning members may in some instances modify their diet advantageously by means of supplementary articles of food secured outside of the household. Finally, there may be mentioned as a possible contributing factor the fact of common observation that the diet of the woman (particularly of the housewife) is more likely to be affected by eccentricities than is that of the man.

Here it may be permissible to suggest that eccentricities in diet probably account for many of the sporadic cases both in the general population and in institutions, particularly institutions for the insane, just as they must account for similar cases of scurvy and of beriberi. This may also explain many, if not all, of the cases in well-to-do individuals although it may be well to point out that it by no means follows that all well-to-do individuals are always provided with a good diet nor that even when so provided that they always eat it. It should not be overlooked that well-to-do people may be food faddists, that they may be parsimonious, or be unfavorably situated with respect to sources of household food supply and, thus, that their diet may be one-sided, restricted and monotonous and possibly faulty in one or more respects (21). So that in studying the dietary relationship of the sporadic case, whether of one occurring in the home or in an institution, the student should not be content with ascertaining the character of the diet available, that is, of the general diet of the household or of the institution, but should strive to determine just what and how much it was that had in fact been eaten by the affected individual. Failure to do this, explains many, if not all, of the cases that have been reported as instances of the occurrence of the disease in spite of a good diet and, thus, as supporting an infective etiology (22).

The fact that the disease occurs in seemingly well-nourished individuals and in nursing infants has also been advanced as an objection to a dietary etiology. It is a sufficient answer to this objection to point to the same occurrence in scurvy and in beriberi.

In the light of the considerations presented it must be clear that the epidemiological and clinical evidence on which the argument for infection has been based either has not the significance that has been attributed to it or is equally consistent with the dietary view.

The argument for infection has not however been based wholly on clinical and epidemiological grounds. A number of workers have advanced either bacteriological (2) or experimental evidence (23) (24) in its support. Of the reports belonging in this category there is only one of sufficient importance to be here considered. This is the report of Harris (24) of New Orleans of having infected two monkeys with pellagra by inoculating a filtrate from pellagrous tissues. Assuming that the diagnosis of pellagra in these animals was correct

there is nothing in the report to show that it was not diet rather than infection that was the responsible factor. But it is by no means certain that the diagnosis was really correct. That it was an error has already been suggested by Funk (25) and is made highly probable, first, by the fact of the frequent occurrence in the monkey of eruptions that so far as may be judged are much like that described and pictured by Harris; second, by the important consideration that neither he (26) nor any other workers (27) have been able to confirm his original results and, third, by the still more important fact that a number of recorded attempts to transmit the disease to the human subject (28) by means of dermal scales, blood, nasopharyngeal secretions, urine and feces from cases of pellagra have been complete failures. The available experimental evidence must be regarded therefore as, on the whole, affording no support for the idea of pellagra as an infection.

It would seem then that, at best, the evidence so far considered either is equally consistent with the dietary hypothesis or actually affords the idea of infection in favor of which it was adduced no real support.

Let us now turn to some observations that, as will be seen, not only afford no support to, but are difficult or impossible to reconcile with an infective etiology. And, first of all, may be cited an observation reported by Külz (29), an army surgeon with the German army of occupation in Roumania during the World War. He has reported that in spite of the closest contact with the Roumanian population, among whom pellagra was prevalent, the several hundred thousand of the military personnel remained free from this disease although they were not exempt from attack by other endemic maladies, such as malaria, typhus, typhoid and dysentery. He characterized this, and, it would seem, not without warrant, as "evidence of very great weight in support of the argument against infection." In this connection, it is of interest to recall that the food conditions in Europe during the World War have been cited by some observers (30) (43) as well nigh conclusive evidence against the dietary origin of pellagra. These observers seem to argue that inasmuch as the people of Europe, particularly those of certain of the belligerent powers, were on starvation diets and necessarily badly nourished during the war and since there was no pellagra amongst them, diet and malnutrition can have nothing to do

with pellagra. So far as concerns the relation of a general malnutrition to pellagra this may be freely granted but it appears to have escaped those who reason in this way that if this argument were really valid as regards diet, it would also disprove the now well established dietary relationship of beriberi for, so far as is known, beriberi was no more prevalent than pellagra under the circumstances mentioned. The fallacy of the argument resides, of course, in the assumption that because a disease is the result of a faulty diet any faulty or inadequate diet will bring it about—a wholly unwarranted yet, curiously enough, a very common assumption. Evidently then the experience of Europe during the war is not inconsistent with the dietary hypothesis.

The experience of the German army of occupation in Roumania, to which reference has been made, in its failure to support the idea of infection is in close harmony with certain older observations the significance of which seems, however, to have escaped or has been misunderstood by the advocates of infection. These observations fall into one of two classes of which the first represents the experience of institutions, both special and general hospitals, receiving cases of pellagra for treatment. In such institutions physicians, nurses, attendants, etc., in frequent contact with the disease and directly or indirectly with the body discharges of persons sick with it, practically never develop the disease while so employed (31). A recent illustration of this is to be found in the experience of Enright (33) with pellagra in Egypt. He states that during the three years that he had dealt with some thousands of cases he was struck by the fact that all the orderlies, including British, Turks, and Germans, in attendance on the patients remained free from the disease. While we can not agree with Enright in considering this as proving that the disease is neither infectious nor contagious, his observation is certainly a very striking illustration of at least the failure of "contact" to operate when given what would seem to have been an excellent opportunity to do so.

Impressive as such observations are, those of the second class are very much more so. This class represents the experience of institutions to which cases of the disease may or may not be admitted but in which it is endemic or has, in some instances, been epidemic, or both. Thus, it has repeatedly been observed that employes (nurses, attendants, etc.) resident in such institutions, many of whom come in

frequent association or intimate contact with active cases of the disease or their body discharges, or both, practically never contract the disease while so employed and so residing (31). This exemption, considering the circumstances, is extraordinarily difficult if not altogether impossible to reconcile with a hypothesis of infection. From the point of view of diet, however, an explanation readily suggests itself and, in fact, in the instances studied by us the exemption was consistently associated with what was believed to be a significant difference in diet. It would appear therefore that not only is the idea of infection without unequivocal support but there is important evidence with which it is difficult, if not impossible, to reconcile such idea but which is consistent with and is readily explained on a dietary basis. Clearly, then, the weight of the evidence so far reviewed, favors the dietary etiological view and more than warrants its adoption as a working hypothesis.

Now if this hypothesis is sound and pellagra is really due to a faulty diet, then certain implications which this hypothesis carries with it must also be true.

These are, first, that a difference in diet as between pellagrins and non-pellagrins be demonstrable; second, that the disease must be curable by a proper diet; third, that it must be preventable by such a diet, and, fourth, that it may be experimentally produced by diet.

The demonstration of a difference in diet as between individual pellagrins and non-pellagrins is frequently difficult or impossible particularly in localities in which the disease is endemic. The reason for this difficulty is, in the first place, the fact that we have no means for determining in a particular instance whether the seeming non-pellagrin is really free from the disease. Experience and analogy with other deficiency diseases both teach that in many instances the disease is very probably present but not developed to the point of symptoms. In the second place, our basic knowledge as to the nature of the dietary deficiency and, more particularly, as to the preventive value of different foods or combinations of foods is, as yet, much too meager to permit of reliable judgment in all instances as to the adequacy of a particular diet. This difficulty is enhanced, moreover, by the indefinite character of the information that is ordinarily available with respect to the diet of the individual. At best all that ques-

tioning can ordinarily elicit is a qualitative statement. In exceptional instances only can even a partial quantitative statement be secured. Manifestly therefore the determination of a difference in such circumstances is not to be expected. If, however, the comparison is not between individuals but between groups a significant difference is demonstrable or may reasonably be inferred to exist. Thus in a study by workers of the United States Public Health Service (34) carried out in some cotton mill villages of South Carolina in which the disease was endemic, comparison of diets of non-pellagrous with those of pellagrous households revealed that the pellagrous had a more restricted supply of such foods as milk and fresh meat. Similarly, in institutions in which the disease is endemic although the non-pellagrous class (consisting of officers and employes) may appear to and, perhaps, actually does receive exactly the same food as does the pellagrous (composed of the inmates) an indication of a difference in the diet of the two exists in this—that those of the unaffected class, being the officers and employes, are in a position both to serve themselves more liberally of the more desirable articles of the ration and to supplement the institution diet with articles of food or with meals secured outside.

Difficult as it may be to discern any difference in the individual diet in pellagrous households in an endemic locality, indications of a difference can very frequently be discovered in sporadic cases particularly in such as occur in well-to-do persons.

Examples of such cases may be found scattered in the literature. Particularly interesting are some of the cases reported by Vedder (17), Roberts (21), Eustis (35) and Wheeler (36), and a case in an army officer reported by Nichols (37). Nichols states that when his case was reported everyone said that this was an instance of pellagra in which the food factor could be ruled out, presumably, because there could be no question of the patient not having had a good diet available; but on going into the history of the case with the patient's sister he found that the patient "had always been a crank on diet, had never eaten meat, and always carried crackers around in his pockets."

If the dietary theory is sound and a difference in diet between pellagrin and non-pellagrin is demonstrable then, it has been suggested, a difference between the diet of a community or locality in which only

exceptional, if any, cases occur and that of one in which the disease is highly prevalent should also be demonstrable. This is a reasonable expectation. Unfortunately, however, the attempt to demonstrate a difference in such circumstances calls for a laborious inquiry not only as to the diet directly but also as to the climatic, economic and other possible conditions that might affect the character of the food supply of a community or locality and thus, indirectly, of the character of the diet of its people. The observers who have reported that a significant difference is not demonstrable (7) (8) (9) (65) have reached this conclusion, we believe, because of inadequate and faulty methods of study of a difficult problem. The more soundly oriented inquiry carried out by Vedder (17) and that by the workers of the United States Public Health Service (6) resulted in adducing evidence that justified at least a strong presumption of the existence of a difference in household diet of contrasting communities. Thus, making due allowance for certain inherent difficulties, indications of a difference in diet as between pellagrin and non-pellagrin whether regarded as individuals, households or communities are frequently demonstrable or may reasonably be inferred.

In this connection the question naturally arises whether the marked annual fluctuations in pellagra prevalence, sometimes observed, can also be associated with fluctuations in diet. It has indeed been argued (67) that it is difficult to believe that the people of any considerable area should make such radical changes in their diet as would explain these fluctuations in prevalence. That this is a hasty and superficial view will at once be realized when it is recalled that quite similar fluctuations in prevalence are characteristic of such dietary diseases as scurvy, and beriberi, so that obviously considerable populations may make or, rather, may be obliged by circumstances to make "radical" changes in their diet. In reality, however, there is no need to assume that the changes in diet must be of a "radical" character. It seems to us quite possible that relatively slight and, superficially, perhaps quite inappreciable quantiative shifts may suffice to change what was perhaps a just sufficient diet into one more or less deficient in some one or more respects. Recalling the intimate relation that exists between economic conditions and pellagra incidence and recalling further that economic conditions are at times subject to violent

fluctuations, one can readily perceive the nature of the force that may oblige the people of a household, of a community or even of a nation to modify their diet for worse or, it may be, for better. The depression in the price of cotton in 1914 consequent upon the outbreak of the war in Europe was followed by a great increase in pellagra in the cotton growing area of the United States in 1915. The recovery in the price of this staple during 1915 was followed by an equally or even more notable reduction of the disease in 1916. Can it be believed that the sharp restriction of credit that the planter or the merchant in the plantation area of the Southern States was obliged to impose on the plantation labor in the fall and winter of 1914 in consequence of his inability to sell his crop without loss was without effect on the diet of those people? No one acquainted with the agricultural economic organization obtaining in the plantation area will have any doubt of the answer.

There is much reason to believe, therefore, that the year to year fluctuations in pellagra prevalence are associated with fluctuations in diet and that these are reflections of fluctuations in economic conditions.

Passing now to the question of the place of diet in the treatment of pellagra, we find that opinion has varied considerably in the course of the long history of the disease but it is worthy of note that over half a century ago Roussel (3), on the basis of his own observations and the experience of others, assigned to diet the place of first importance.

The testimony of many careful observers and the results of recent studies all go to show that in uncomplicated cases of average severity the administration of a diet containing a generous allowance of milk and fresh meat is quite regularly followed by clinical improvement. With only rare exceptions, provided the patient coöperates fully in taking the diet, this improvement continues progressively to eventual complete clinical recovery without the use of any medication except such as may be needed to mitigate distressing symptoms or complications. Should the coöperation be unsatisfactory or the diet for any reason be inadequate, the attack takes a very different course: in the milder attacks the manifestations, particularly the eruption, may clear up and the attack may seem to subside. But such seeming improvement is temporary. After a variable period (possibly of weeks) the

manifestations reappear perhaps again to subside but sooner or later these remissions cease. In other, as a rule more marked, cases no remissions occur, the attack with or without a period of seemingly stationary symptoms becoming progressively more pronounced and severe. Even at such more advanced stage a favorable change in food intake may be followed by improvement and recovery. It would seem clearly indicated therefore that diet operates as a dominating factor in controlling the clinical course and outcome of the attack. A conclusion that is strengthened by the fact that there are no observations however discordant they may have been thought to be that are really inconsistent with it (38).

That the specific treatment of pellagra resides in diet is further and more convincingly shown by the fact that the clinical recovery obtained by proper feeding is maintained as long as a proper diet is continued. In other words, recurrence of the disease so long dreaded by both laity and physician does not take place in those adequately nourished. This was for the first time demonstrated by studies carried out some ten years ago by Goldberger, Waring and Willets (39), and has since been confirmed by other workers (40) (41).

Not only was it demonstrated in those studies that the disease does not recur in those previously attacked but, still more, that it does not occur at all in those subsisting on an adequate diet. Since care was taken to make no changes in sanitary and hygienic conditions, nor as regards the character of new admissions to the institutions in which those studies were made, the complete prevention of both recurrent and new cases proved conclusively that pellagra may be prevented by diet without the aid of any other factor, hygienic or sanitary. And, it may be added, there is no good evidence that the disease can be prevented by any other means.

Not only has it been demonstrated that the disease is completely preventable by diet but, what is more, the production of the disease by diet has also been shown to be possible. In 1915, Goldberger and Wheeler (16) carried out an experiment at one of the Mississippi penitentiaries in which by feeding a diet resembling such as in previous observations they had found associated with a high incidence of the disease they succeeded in producing the disease in definitely recognizable form in at least six of eleven volunteers, all white male con-

victs. As controls they had the other residents of the penitentiary, 120 in all, of whom 47 were under observation throughout the experimental period. None of the controls developed any recognizable evidence of the disease.

This experiment has not escaped criticism. Shortly after the appearance of a preliminary report, MacNeal (42) (43) questioned its validity by casting doubts on the diagnosis of the experimental cases. MacNeal's criticism has recently been echoed by Merk (66) of Innsbruck. Referring to MacNeal's criticism, Wilson (44) of the School of Medicine of Cairo, Egypt, says: "MacNeal casts doubts on the correctness of the diagnosis; no one, however, with some experience of the disease can fail to be convinced, after reading Goldberger's recent paper, that these men suffered from pellagra; the writer from recent experience in Egypt would be inclined to believe, from the peculiar lingual and intestinal symptoms described, that four others might well have been included as pellagrous, leaving one only out of the eleven who does not appear to have become affected." If it be permissible to amend Professor Wilson's comment we would suggest that to be convinced that these men had pellagra, one other requirement must be fulfilled—namely, the possession of an open mind.

Although relatively great progress has in the past ten years been made in our knowledge of pellagra, this progress has been slow when compared with that recently recorded in connection with certain other diseases of nutrition. The difference is largely if not altogether due to the fact that for pellagra an experimental animal has been lacking. There is reason to believe, however, that this deficiency has now been supplied.

In August, 1917 Chittenden and Underhill (45) reported the experimental production in dogs, by feeding, of a pathological condition which they regarded as closely resembling human pellagra. Wheeler, Goldberger and Blackstock (46) have identified this experimental condition with the spontaneously occurring disease of dogs known to American veterinarians as black tongue. It is of interest to note that the resemblance of the spontaneous disease to human pellagra had been commented on by at least one observer (47) even before Chittenden and Underhill's publication. More recently Underhill and Mendel (48) have briefly reported some results of a study of this con-

dition begun in 1918 and not yet completed. They state that the syndrome in the dog is associated with a lack of some unknown constituent of butter fat which is different from fat soluble A since cod liver oil does not protect. They have reached the conclusion also that fresh beef possesses some protective action but in the absence of a sufficiency of the unknown effective agent present in butter the disease may be induced on a diet containing much meat but a longer period of time is necessary. The same statements are reported as applicable to casein which has been purified by boiling with alcohol. They have found also that egg yolk confers a certain degree of protection but is not as effective as butter fat. Carrots are reported as particularly effective in alleviating the syndrome when it is once initiated. In a paper that has just appeared Goldberger, Wheeler, Lillie and Rogers (49) report in a preliminary way, some results of a study of experimental black tongue that suggest very strongly that this condition and human pellagra are etiologically identical. They have induced this canine disease by feeding dogs certain diets previously found associated with the occurrence of pellagra, including the diet used in the human experiment carried out at a Mississippi penitentiary. Based on their experience with pellagra they have constructed a diet with which they are able regularly to induce this disease in the dog in from one to three or four months. They report that substances such as yeast, yeast extract and fresh lean beef, that were found to possess black tongue-preventive potency, have when tried in pellagra been found efficient preventives of the human disease and, conversely, that the substances such as butter fat, cod liver oil and casein that had failed in pellagra or that, like milk, were of low pellagra-preventive potency, when tried in black tongue failed or were feeble as preventives of the canine disease. In consequence of these striking findings they have adopted the working hypothesis that black tongue of dogs is the analogue of pellagra in man.

In the same publication these workers also report results that seem to indicate that the rat too will probably be found to serve as an experimental animal in the study of pellagra and of black tongue. We shall return to this presently in another connection.

Thus it would seem that we are warranted in concluding not only that indications of a difference in diet between pellagrin and non-

pellagrin are demonstrable and that the disease is curable and completely preventable by a proper diet but also that it may be experimentally produced by diet in man and with a high degree of probability (its analogue) in the dog, and possibly the rat, thus proving that the implications of the dietary hypothesis, so far as they have been tested, are true. Accordingly, in the light of the indications afforded by the evidence previously considered it may be concluded that diet is the primary controlling factor in the causation of the disease.

Accepting this conclusion the question now arises as to the nature of the essential dietary fault upon which the production of the disease depends. Needless to say, many suggestions along this line are to be found in the voluminous literature. The older of these, such as that of quantitative inadequacy, of monotony of diet, of monophagism, of spoilage or, specifically, of spoiled maize, and of carbo-hydrate excess have all been shown to be untenable. We may therefore pass on to a consideration of those more recently advanced. But even of these only the most immediately pertinent can for obvious reasons be considered.

We shall begin with the views advanced by Funk (50). In 1912 this worker provisionally included pellagra in a group of "deficiency diseases," all of which, he stated, could be prevented and cured by the addition to the diet of certain preventive substances called by him "vitamines."

Interesting and stimulating as this generalization has proved to be he advanced in its support, so far as pellagra is concerned, only certain epidemiological and clinical analogies to beriberi. More recently (51) he has expressed himself as inclined to the view—which he not altogether correctly credits to Goldberger—that the following factors are possible: "Partial lack of vitamines; lack of animal protein; lack of a still unknown vitamine; the combined influence of all these factors."

Inspired by Funk's and the other then recent developments in beriberi, Sandwith (52) in 1913 suggested that pellagra might be a "deficiency disease waiting for a vitamin to be discovered."

As the result of extensive studies, Voegtlin (53) in 1914 expressed the opinion that in the study of the etiology of pellagra serious con-

sideration would have to be given to a deficiency or absence of certain vitamines in the diet, to the toxic effect of some substances, as aluminum, which occur in certain vegetable food and to a deficiency of the diet in certain amino acids. Later, from a study of the influence of vitamines on the clinical course of pellagra, Voegtlin (54) in association with Neil and Hunter reported that the administration of extracts from yeast and rice polishings which were highly efficient for the prevention of avian polyneuritis, in general failed to modify the course of the disease, but the administration to pellagrins of protein-free extracts obtained from liver and thymus gland, presumed to contain both the antineuritic substance and the fat soluble vitamine, "was followed by improvement in their condition apparently comparable to that produced by the consumption of a diet rich in fresh animal proteins." They concluded that "the dietary defect responsible for pellagra is distinctly (qualitatively) different from and perhaps more complex than the one causing fowl polyneuritis and human beriberi."

In 1918 McCollum, Simmonds and Parsons (55) on the basis of results of studies in rats, expressed the belief that pellagra is primarily associated with the unsatisfactory character of three dietary factors, namely, fat-soluble A, mineral elements and protein mixture. A year later, after having attempted to produce in rats a condition analogous to pellagra in man by feeding with diets similar to the diet employed by Goldberger and Wheeler in their experiment in convicts, and having observed in them only a "generalized poor condition," they (56) concluded that pellagra is caused by an infectious agent. More recently McCollum (57) has stated that "it seems established that pellagra is in some manner caused by faulty diet," but expresses no opinion as to the nature of the fault.

As the result of studies of diets of various groups, pellagrous and non-pellagrous, carried on in Egypt Wilson (44) reported in 1921 that the etiological factor is a deficiency of protein in the food best determined by an estimation of its biological value by means of Thomas' figures.

In 1920 Wood from some experiments with fowls, and by reason of seemingly favorable results of treatment with maize germ and wheat bran, reported that he was disposed to suspect that a vitamin-B

deficiency is involved in pellagra but pointed out, at the same time, that there may be something else in the maize germ and the cortex of wheat that may account for the results observed.

Beginning their investigation of pellagra in 1914, Goldberger and associates have reported a series of studies the results of which they have interpreted as permitting the exclusion of one after the other of the known dietary essentials—until in 1922 they (58) concluded, on the assumption that all the dietary factors essential in human nutrition were known, that the essential etiological dietary factor is a specific defect in the amino-acid supply, probably in the nature of a deficiency of a special combination or combinations of amino acids. Realizing, however, that the assumption that all dietary essentials were known was of doubtful validity (59) but considering that as between the protein and an as yet unrecognized factor the probabilities favored the former as being the one concerned, they undertook a study of the therapeutic and preventive value of the protein of casein late in the summer of 1922. Early in 1925 they (60) reported that while the casein had had a beneficial effect on the general nutrition of their patients and in considerable measure prevented or, at least, notably delayed the development of the distinctive eruption, it did not prevent, though it may have delayed the relapse or recurrence of some of the other symptoms of the disease. They concluded therefore that the protein factor, if it be concerned in pellagra is not the sole preventive or causative factor and thus that some other heretofore unrecognized or unappreciated dietary complex also plays an essential rôle. This interpretation was supported by the results of a preventive study of dried skim milk when contrasted with those of a study of fresh buttermilk; on the basis of protein content dried skim milk proved itself distinctly inferior to fresh buttermilk. It was supported still more, perhaps, by the results of a study of the pellagra-preventive potency of dried yeast. In a daily dose of between 15 and 30 grams, representing less than 15 grams of protein, dried yeast was found very efficient in preventing the disease, a result that in view of the proved inadequacy of casein it was difficult or impossible to credit to its protein alone. They thought too that in view of the relatively small amount of protein furnished by the effective dose of yeast the special pellagra-preventive factor, which they designated as factor P-P,

would seem capable of preventing the disease with little if any co-operation from the protein and, further, that it is possible that factor P-P plays the sole essential rôle in the prevention and causation of the disease.

This interpretation is strengthened by the results of further studies just reported by these workers (49). In tests of fresh lean beef and a commercial dried water extract of yeast they found both efficient pellagra-preventives. In this study the daily allowance of beef was 200 grams (7 ounces) and of the yeast extract 15 grams. In view of their previous experience with casein the protein of which in a daily allowance of 60 grams had been found not fully adequate entirely to prevent the disease, it is reasoned that since the allowance of beef yielded but about 45 grams of protein and that of the yeast extract could not have contributed over an equivalent of about 7.5 grams of protein, the potency of the beef and that of the yeast extract could not well be attributed to their protein alone. They therefore concluded that lean beef and yeast extract contain factor P-P and that this factor, in view of the effectiveness of the yeast extract with its small yield of protein, probably plays the primary rôle in the prevention and causation of pellagra.

They point out that the demonstration of the pellagra-preventive potency of yeast and yeast extract which, aside from protein and a negligible trace of vitamin A, has heretofore been believed to contain only the water soluble B vitamin naturally raises the question of the relation of factor P-P to vitamin B. They recall that although "water soluble B" has quite generally been considered as representing a single dietary factor with both antineuritic and growth-promoting properties (61), a number of investigators (62) have not accepted this view; inclining to the belief that it includes at least two distinct dietary essentials, namely, the antineuritic or beriberi vitamin to which alone, following Funk, the designation "vitamin B" belongs and a "growth-promoting" factor which some workers have attempted to identify with bios (63).

That vitamin B in the sense of the beriberi vitamin or antineuritic is not concerned in the etiology of pellagra or, in other words, that it is distinct from factor P-P and that these two factors may perform their physiological functions one independently of the other is, they

state, even aside from any other evidence, quite conclusively shown by the rarity of association of beriberi and pellagra. The fact that these diseases in rare instances do occur together in the same patient in their judgment only emphasizes the significance of the rarity of such cases. Thus although the diet may be deficient in both the beriberi and pellagra-preventive essentials, ordinarily in relation to these diseases it is deficient in one and not, or clinically but inappreciably, in the other factor.

With respect to the relation of factor P-P to the so-called growth-promoting essential, heretofore possibly included in the term "water soluble B," Goldberger and associates bring forward evidence which seems strongly to point to their probable identity.

They state that having found yeast to contain the factor concerned in the prevention of black tongue and of pellagra, and having assumed, for reasons already referred to in a preceding connection, that this factor is the same for both conditions, they undertook a study of the characters of this factor as it exists in yeast. By means of black tongue-preventive tests in the dog they found that factor P-P is adsorbed from an acidulated water extract of yeast by English fullers' earth (Seidell's activated solid); that it is destroyed by charring the yeast but is damaged but little, if at all, when the yeast is heated in the steam autoclave at fifteen pounds pressure for $2\frac{1}{2}$ hours. They report further that when young rats are fed a diet that is complete for growth except as to "water soluble B" and contains as the sole source of this vitamin as much as 30 or 40 per cent of such autoclaved yeast (Chart 1; Chart 2, period 4), their growth is quickly arrested, then their weight declines and they die with or without symptoms of polyneuritis, although the same yeast before autoclaving when forming 8 or 10 per cent of the diet furnishes sufficient "water soluble B" for good though not optimal growth. Thus, they point out, according to current ideas the autoclaving does not notably affect factor P-P but (in yeast) inactivates the "water soluble B." Evidently, too, factor P-P is not of itself growth-promoting in the rat. Furthermore, if the so-called growth-promoting water soluble vitamin of the yeast is distinct from both the antineuritic and the P-P factor, then clearly either the heating has inactivated it or like factor P-P it is not a special growth factor.

But that P-P or some associated, and in yeast relatively heat resisting, factor is essential for the growth of the rat is strongly indicated by the results of some work with autoclaved yeast recently reported from the Hygienic Laboratory by Smith (64) and clearly shown by Goldberger and associates by the following:

1. Young rats were fed a diet complete for growth except as to "water soluble B" and containing as the sole source of "water soluble B" as much as 30 or 40 per cent of autoclaved yeast which from tests in dogs had been shown to contain factor P-P; the rats quickly declined in weight, after a slight initial rise, and died with or without

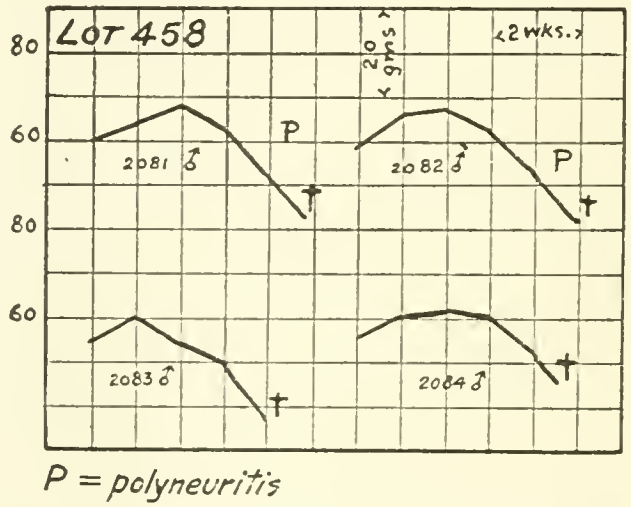

CHART 1. Weight curves of four young albino rats (Lot 458) whose diet (No. 227) included as the sole source of "water soluble B" 27 per cent of autoclaved yeast. Slight initial growth was followed by arrest and continued loss of weight terminating in death. Two of the animals developed signs of polyneuritis. (After Goldberger, Wheeler, Lillie and Rogers.)

signs of polyneuritis, thus giving proof of an antineuritic deficiency (Chart 1; Chart 2, period 4).

2. Young rats were fed a diet complete for growth except as to the "water soluble B" but containing as the sole source of this vitamin as much as 40 per cent of a preparation of an alcoholic extract of corn meal (Chart 2, period 1) that was found potent in alleviating or curing polyneuritis in the rat. The animals notwithstanding an abundance of antineuritic quickly declined in weight after slight initial growth.

3. When, however, young rats were fed a diet as before complete for growth except as to "water soluble B" but containing as sources

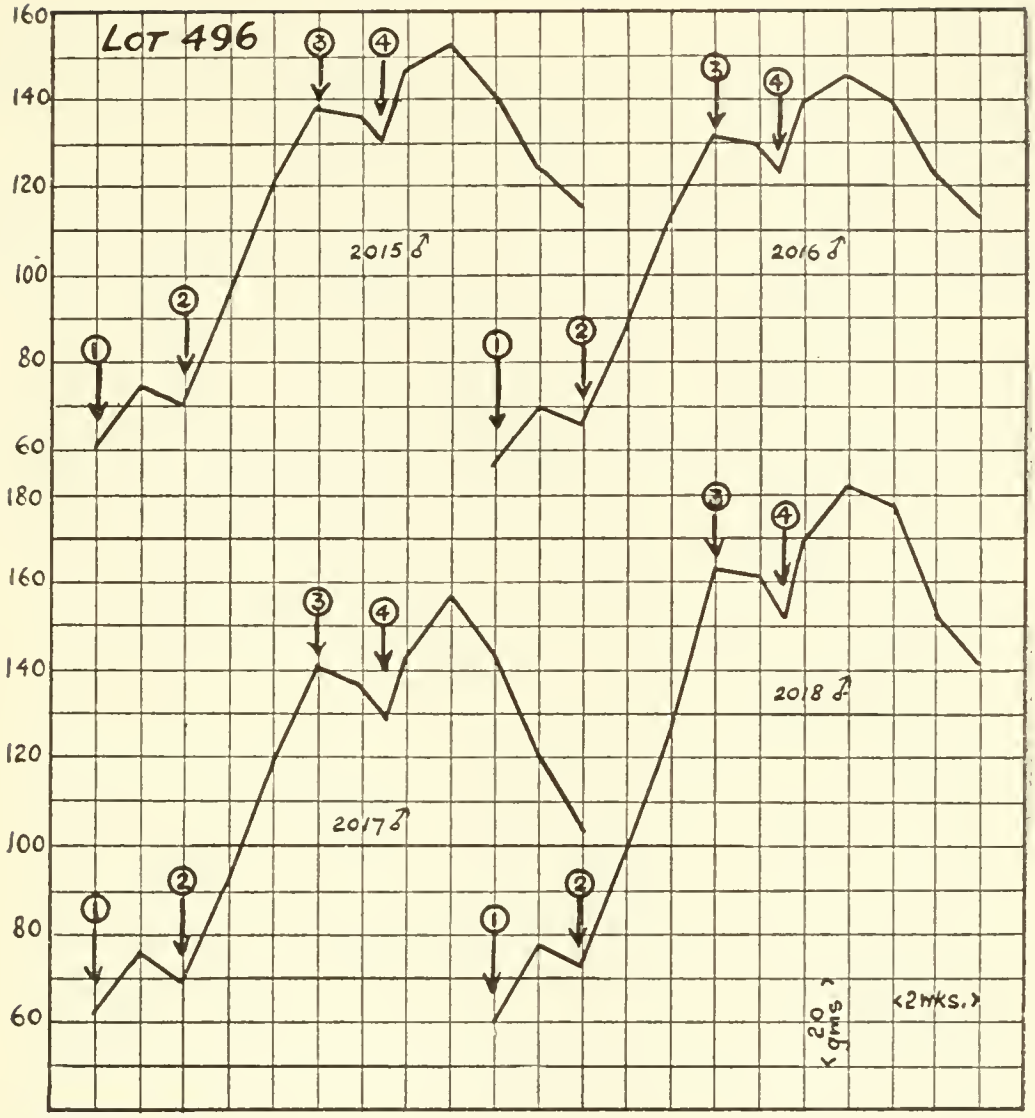

Chart 2. Weight curves of four young albino rats (Lot 496) during four dietary periods. During period 1 their diet (No. 218) included as the sole source of "water soluble B" 40 per cent of an alcoholic extract of corn. After an initial gain in weight they began to lose, whereupon there was added to their diet 9 per cent of yeast previously autoclaved at 15 pounds for 2½ hours. This was at once followed (period 2) with a resumption of growth which was well maintained during three weeks at the end of which a change in diet was again made. This change consisted of the withdrawal of corn extract and autoclaved yeast thus giving them the basic diet (No. 206) without any known source of "water soluble B." Growth was at once arrested followed by a downward trend in weight (period 3). Now another change in diet was made. The basic diet (No. 206) was replaced by one which included 40 per cent of autoclaved yeast as the sole source of "water soluble B" (diet No. 239). This change was followed by a resumption of growth which lasted but a short time and was followed by a progressive loss in weight. Thus neither 40 per cent of the corn extract nor 40 per cent of the autoclaved yeast when the sole source of "water soluble B" permitted the rats to grow but when only 9 per cent of the autoclaved yeast was added to the diet containing the corn extract growth took place and was maintained. (After Goldberger, Wheeler, Lillie and Rogers.)

of this vitamin as little as 8 or 10 per cent of the autoclaved yeast and as little as 5 per cent of the preparation of the extract of corn meal, the animals grew (Chart 3; Chart 2, period 2; Chart 4).

Again, when young rats were fed a diet complete for growth except as to the "water soluble B" and containing as the sole source of this vitamin 20 per cent of dried fresh lean beef which, judging by ex-

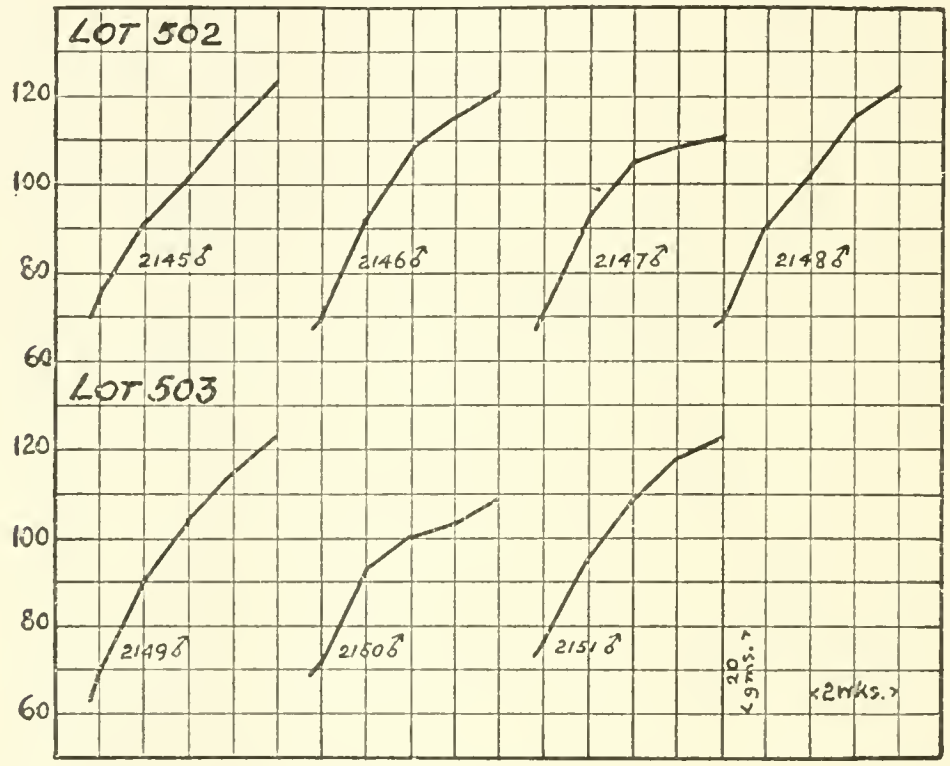

CHART 3. Weight curves of two lots of young albino rats. The diet of both was free of "water soluble B" except as supplied by 5 per cent of a corn extract combined with, in the case of lot 502, 8 per cent (diet 243E) and in the case of lot 503, 10 per cent (diet 243F) of autoclaved yeast. Although neither preparation alone when the sole source of "water soluble B" even to the extent of 40 per cent of the diet permits growth when, as here, much smaller proportions of each are combined growth takes place thus proving conclusively that this is not simply an additive phenomenon. The growth of these animals is at a somewhat reduced rate; for optimal growth the percentages of both corn extract and autoclaved yeast would have to be increased. (After Goldberger, Wheeler, Lillie and Rogers.)

perience in both black tongue and pellagra, contains factor P-P, such animals after slight initial growth declined in weight and died with or without polyneuritis, thus indicating an antineuritic deficiency (Chart 5, period 1). When, however, on the appearance of signs of polyneuritis there was included in the diet of these animals as little as 5

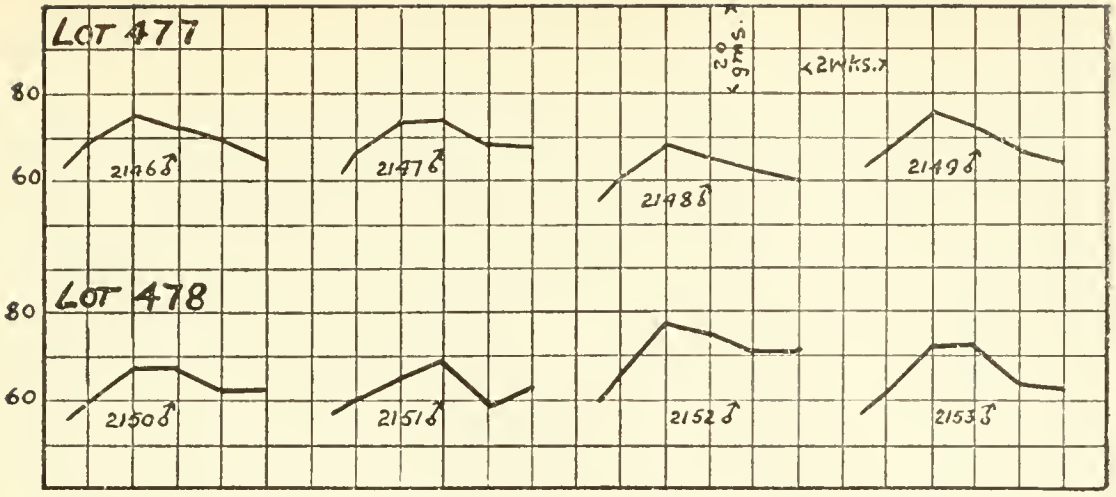

Chart 4. Weight curves of two lots of young albino rats. The diet of both was free of "water soluble B" except as supplied by 6 per cent (Lot 477, diet 238B) and 12 per cent (Lot 478, diet 238C) respectively of a corn extract. Growth was quickly arrested. (After Goldberger, Wheeler, Lillie and Rogers.)

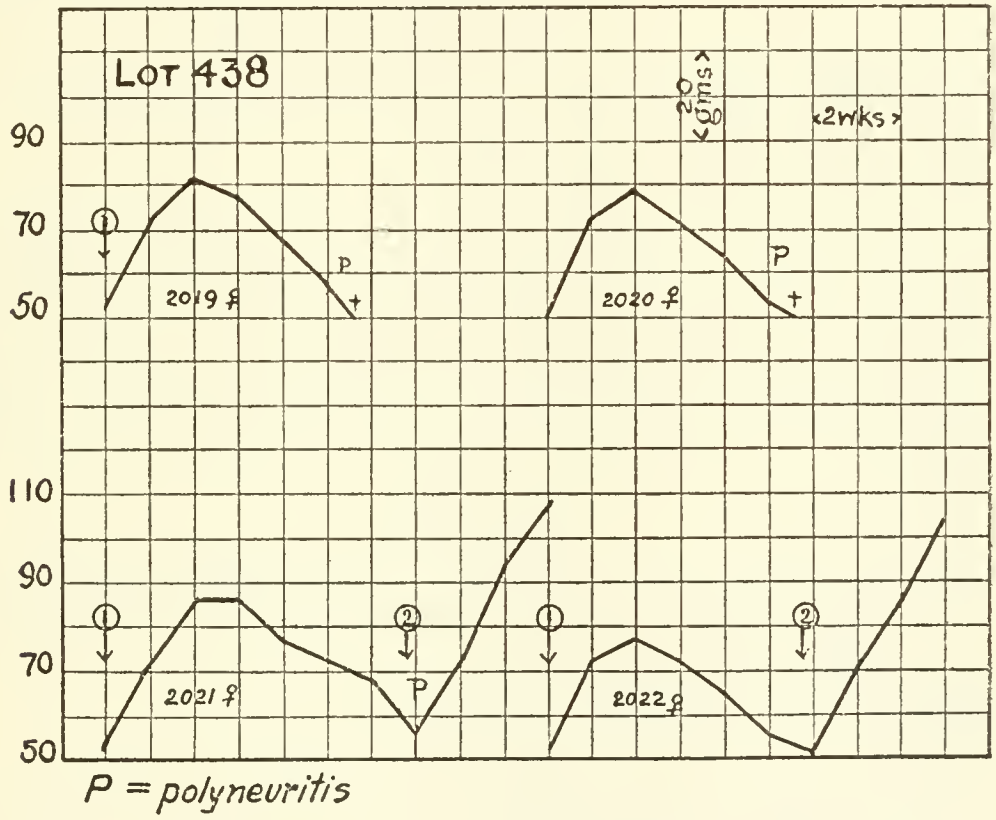

Chart 5. Growth curves of young albino rats (Lot 438) whose diet during period 1 included as the sole source of "water soluble B" 20 per cent of dried fresh beef. After some initial growth there was arrest followed by loss of weight with the development of signs of polyneuritis in three of the animals. After two of these died the diet was modified by adding 5 per cent of our corn extract (diet 219A). This was followed by disappearance of the signs of polyneuritis in the survivor showing these, with prompt resumption of growth in both survivors (period 2). The beef contained sufficient P-P but was deficient in antineuritic. The small addition of corn extract supplied enough of this to supplement P-P sufficiently to permit growth to take place. (After Goldberger, Wheeler, Lillie and Rogers.)

per cent of the preparation of alcoholic extract of corn, 40 per cent of which as the sole source of "water soluble B" in a diet does not enable the rat to grow, the animals that were not too far gone recovered from polyneuritis and resumed growth (Chart 5, period 2). Clearly the alcoholic extract of corn although capable of curing polyneuritis in the rat does not of itself promote growth but does permit or promotes growth when combined with a suitable proportion of a P-P containing substance, such as autoclaved yeast or beef, which itself, within certain limits at least, neither prevents polyneuritis nor permits growth.

Thus autoclaved yeast and beef muscle were shown to contain a factor distinct from the polyneuritis-preventing essential which, in combination with the antineuritic, is necessary for the growth of the rat. This factor which is perhaps identical with the yeast growth-stimulant or bios is at present indistinguishable from factor P-P; but whether the two are really the same, as seems very probable, will require further study to determine.

Summing up, it may be stated that the available evidence seems to leave no reasonable doubt but that pellagra is caused by a faulty diet. The primary dietary fault appears to be of the nature of a deficiency of a factor P-P, very probably but not certainly identical with a dietary essential, heretofore included with the antineuritic under the designation "vitamin B", which some workers have attempted to identify with bios.

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CONSTITUTION AND TYPE IN RELATION TO DISEASE[1]

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From almost the beginning of medical history the rôle of constitution in relation to disease has been recognized, yet in spite of the age of this problem very little accurate scientific knowledge is available bearing on an exact connection between a definite constitution and the nature of its diseases.

Ultimately no doubt this problem must require much statistical and biometrical control but in its present phase considerable general morphological data are much needed. It is certainly equally as important to ascertain what to measure as it is to make the measurements. Undoubtedly much time and effort have been fruitlessly spent in making many careful measurements of meaningless characters. One is certainly highly optimistic who can survey the enormous accumulation of anthropometric figures and indices without feeling a considerable degree of disappointment over the almost complete absence of explanation or analysis of the causal elements in racial differentiation. The simple and very significant differences in cephalic index have scarcely given the slighest clue to the cause of head shapes or to racial relationships. The quite distinct anthropological differences between the two well recognized human types have indicated practically nothing as to their real nature or origin. Many anthropologists have considered head shape a most important racial feature yet if one should use such a criterion in classifying the various breeds of dogs he would become considerably confused. The dolichocephalic head of the Esquimo and the brachycephalic head of an achondroplastic man should cause some uncertainty regarding the use of head index as a racial criterion. This is not to be taken as a criticism of the anthro-

[1] DeLamar lecture delivered before the School of Hygiene and Public Health, Johns Hopkins University, March 15, 1926.

pometric method in itself, but the way in which it has been employed has not proved very instructive.

During the past few years there has been a decided revival of interest in the study of the relation between constitution and predisposition to disease. However, in presenting the subject I have thought it best not to attempt a discussion of the specific problems on which we are at present engaged, and from which only very partial and somewhat indefinite results are available but, rather, to devote the present consideration to the general problem of constitution and the methods by which this problem may be definitely approached and possibly analyzed.

In the first place, before we are able to understand the relation of constitution and type to disease, it would seem necessary to know exactly what "type" and what "constitution" actually mean. As I consider the terms, type and constitution are not synonymous. The type indicates the fundamental character of the individual and remains fixed throughout life, while the constitution of the same individual changes during different life periods and may readily be greatly modified at any time. For example, a linear type individual may during adult life develop mild acromegalia—this does not alter the type though the constitution of the person becomes greatly modified. If pure types do exist, naturally type hybrids also occur. What are their characteristics and how are they to be recognized and classified? Probably a great deal of the uncertainty surrounding the connection between constitution and disease results from the fact that there is no clean cut agreement as to what the different constitutions actually represent. From the medical standpoint scarcely anyone has sought to determine the basis or origin of a definite type or constitution. There must be some uniform criteria for contrasting the several kinds of constitutions, and before this is clearly determined it is difficult to proceed. An accurate study of constitution itself seems to me the problem of first importance.

The general study of the whole subject has, however, largely proceeded the other way around. Physicians interested in disease have more or less intuitively come to believe that certain types of people do exist and seem predisposed to particular kinds of diseases. There is often a pronounced diathesis on the part of a given patient towards

particular diseases. There is, for example, the tubercular tendency. Many discerning physicians are more or less successful in recognizing these tubercular disease types. But when the uninitiated seeks a description of the particular type it is most difficult, if not impossible, to obtain it except in vague, general and indefinite terms, and in almost no cases has serious study been devoted to the very important question of the nature, origin or development of the given type or to the very fundamental question,—whether an actual type is really concerned.

What is there peculiar about the composition or constitution of the particular patient which causes him to be subject to tubercular disease in contrast with the more resistant individual? Is this difference genetic or developmental in origin, or both, or is it due to differing degrees of an acquired reaction on the part of persons who have been subjected to some definite and peculiar environmental stress or condition? The answers to such questions as these are certainly necessary for a better understanding of the problem.

The physician studying constitution frequently confines his attention to only one life period of the individuals involved, and usually to the most static period, the adult. Dr. Draper in his recent anthropometric study of certain disease groups has confined his material to persons between the ages of eighteen and sixty. Such restriction eliminates almost all growing and developing individuals as well as many of the important late senility conditions. A study of such groups leads one to overlook arrested and childlike tendencies in particular persons as well as many abnormal secondary growth reactions. The rare occurrence of a typically adult disease among children may furnish a simpler clue to the constitutional diathesis than the study of great numbers of adults with this disease. Deviations and odd conditions often give the plan away more decidedly than routine happenings.

Constitution itself is not a static or fixed affair but is a developmental condition depending upon the genetic composition of the individual to a large extent, but also depending in a most important degree on the manner of development and growth. The constitution of a given individual is actually a different thing at different life periods. In this way constitution is to be differentiated from type

which, as a rule, is characteristic and true for the individual throughout life—type is, as it were, the static element which underlies the changing constitution.

The prenatal period is probably the most important in determining all later constitutional conditions since at this time the actual gross body form and structure is being laid down and any environmental peculiarity may exert a marked influence on the course of development and may actually determine whether the individual will develop in a normal well-ordered fashion or into a deformed, crippled and deranged specimen. This prenatal stage, although probably the most important and the one on which all subsequent life stages directly depend, has been largely neglected and is almost unknown to the majority of physicians. A clearer understanding of the relation of constitution to type and to disease may be actually obtainable through a consideration of the embryonic and foetal development which has produced a given individual.

The constitution of babyhood is quite different from that of childhood. The undifferentiated and undeveloped baby reacts differently to the various types of food and other stimuli from the way in which the child does. The constitution of the child becomes considerably revolutionized at the time of puberty and the general reaction at this time is not entirely predetermined but may be modified by various environmental conditions; and youth, after puberty, passes through a stage of growth and maturity which largely depends on the success of the puberty changes themselves. Much is to be appreciated regarding the controlling elements in constitution by a study of these important changes. The final adult constitution is therefore a somewhat uncertain accomplishment which has been affected and influenced by both internal and external factors that have acted on the organism from the early stages of development until the final adult condition has been reached. Through all these various age periods of the constitution there exist distinct type differences which separate one group of age constitution from another.

Certain races tend to show characteristic constitutions which may differentiate them from other races in various ways, but aside from the general tendency of a well marked race to exhibit a more or less uniform racial constitution, there are individuals among all races which

show widely divergent constitutions, differing entirely from the average pattern. Such individuals are either due to developmental deviations from the general pattern or to genetic modifications, and they are very important in analyzing the nature of the normal race pattern.

From the above, as indicating the general situation, it becomes evident that we must determine whether different types actually do exist and what are their natures and general characteristics, since on these type differences constitution largely depends. A ready way to proceed may be to review in a general manner the information obtained from a study of lower forms. If types exist among the human population, it is quite probable that similar types also occur among other mammalian forms, particularly the domesticated animals.

I have been led to believe during the past few years that certain domestic mammals, such as dogs, exhibit in a pronounced way, a great many of the so-called types which have been recognized among men. In these lower forms many such types have actually been selected and preserved for centuries in some cases, and now constitute rather typical breeds or races of animals. The structural, physiological and psychological characteristics of a given breed are quite definitely maintained. By attempting to analyze these breeds through a comparison with certain experimental results, as well as with distorted developmental conditions, I believe we shall succeed in getting important information for a more logical understanding of the human conditions.

Before considering the types themselves we may briefly review the possible hereditary background of some exaggerated conditions in relation to certain disturbances and changes in the germ plasm. It has long been recognized that certain germ-cells in a normal parent may be weak and defective and it frequently happens among apparently good stock that peculiar genetic abnormalities arise. A very striking case in point has recently been presented by Sewall Wright from genetic studies on guinea pigs. A family of guinea pigs from ordinary stock began to produce a more or less definite abnormality affecting the development of the lower jaw and the position and development of the ears, a condition of otocephaly. The lines giving this peculiar condition were definitely recorded and all such animals could be traced

back to a single brother-sister mating. The descendants from this mating, however, showed very different tendencies. Certain branches arising from the common ancestry failed entirely to show any abnormal development, while in other branches of the family a very high percentage of otocephalic individuals occurred in one generation after another. Dr. Wright found, on analysis, that this condition was transmitted as if multiple factors or a number of genetic units or genes were involved and the abnormality had probably arisen through some spontaneous modification in the genetic composition of the animals. The defect itself, however, is of the nature of a typical development arrest and without knowledge of the breeding records one would be inclined to interpret this situation as due to some developmental disturbance rather than to an heredity cause.

Modifications closely comparable to the above have been produced in experimentally treated animals. Certain chemical treatments when administered to guinea pigs and other animals seem to affect some of their germ-cells in a general way, causing these to give rise to poorly developed or actually abnormal individuals. The constitution and probably the type of these individuals are decidedly modified and differ from those of the general stock. An interesting result of this kind has recently been reported by Bagg and Little. Mice from well-known stock were subjected to short treatments by x-ray. The first few generations of descendants from these treated animals were quite normal but during later generations very peculiar abnormalities appeared and, by selection, it was possible to secure animals which produced offspring everyone of which showed underdeveloped and abnormal eyes. Among the same group of animals an abnormal kidney condition occurs, some individuals showing a complete absence of both kidneys while others may have abnormal kidneys or may have one normal kidney with the other entirely absent. A line of animals has been selected which breeds almost 100 per cent pure for kidney defects. Whether these peculiarities were actually induced by the x-ray treatment or not is at present impossible to say since the experiment has not been successfully repeated, but for our present consideration this is unimportant. The fact of real interest is that a peculiar genetic composition has arisen in this race of mice which causes them to produce peculiarly defective specimens. The constitution of such

individuals is highly modified and they are certainly subject to definite structural diseases which are extremely rare among the stock population in general. A few other cases might be cited to show that the hereditary material itself may possibly become altered or modified so as to give origin to peculiar developmental processes which ultimately predispose the individual to very definite types of physical disease.

The nature of many of these defects which are definitely found to be germinal in origin is surprisingly similar to other defects which are induced by simply arresting the development of a typically normal embryo. In other words, the germinal modification seems to have caused simply a lowered developmental potential rather than to have altered any particular genetic character. When embryos are arrested at exact stages of development certain organs and parts are inhibited and modified in their structural development. It has been clearly demonstrated that these defects, which definitely modify type and constitution, are induced by disturbed development. The most convincing proof of this is shown by the frequent differences in developmental success attained by the two components of a double monster or united twin individuals. It commonly happens among joined or united twin individuals that one component is arrested or handicapped in its development while the other develops perfectly normally. In this case there can be no doubt that the abnormality of one individual is solely due to some developmental disturbance since the genetic basis of both components must necessarily be identical. These examples of general modification resulting from disturbed embryonic development are of course quite monstrous and exaggerated in their condition, yet I believe that milder expressions of many such conditions as these are most important elements in the general determination and development of the different kinds of individuals among the normal population. Various human types, as well as the types among lower animals, should be clearly recognized as definite growth reactions which result from slight distortion in either the internal or external factors which affect development. Among extreme cases it is often easy to demonstrate this fact and also to show something as to what the cause has been. Since as a rule an almost complete series of gradations exists from these extreme cases, up to the really normal individual, we are

finally justified in presuming that the slight deviations may be of the same general causal origin as are the more pronounced gross deformities.

We may now examine some of the more usually recognized and accepted types and shall again start with rather extreme forms. All physicians recognize several classes of dwarf persons. The best known and at present probably the best understood dwarf is the ordinary thyroid cretin. This individual in the typical case is largely infantile and almost completely arrested in a state of development comparable to early childhood. The condition is definitely known to be due to an extremely defective or absent thyroid gland, and is very characteristic and readily recognized in spite of the various racial groups or even animal species among which it occurs. It would seem to be a definite developmental affair. There is no genetic basis necessarily concerned yet it is not at all impossible that such conditions might arise from definite germinal causes.

Other types of dwarf, such as midgets and achondroplastics, are probably of somewhat more complex origin, and such dwarfs exist in typical form among dogs and among other of the lower animals. There is an hereditary background for their condition and such animals may be bred with a high degree of constancy, yet the expression of the condition is quite variable and rigid selection is constantly necessary to maintain a fixed quality of the stock. The King Charles Spaniel and the Pekingese breeds of dogs are midgets with considerable achondroplasia, and these also show rather marked exophthalmic conditions. The thyroid and other glands of internal secretion in these dogs show certain peculiarities in their histological structure and the function of the glands is considerably disturbed. The general modified structure and shape of the animal results very probably from these modified internal secretions, and yet the peculiar condition of the glands of internal secretion is very likely inherited in either a direct or secondary way. In other words, it seems at present that these dog breeds actually inherit a peculiar gland modification and, as a result of this modification, their entire growth pattern is distorted and modified. Their physiological and psychological performance is also peculiar, and quite characteristic of the definite individual type or breed. The breeds of dogs are known as groups to be either gentle or shy,

kind, or irritable and snappy, stupid or intelligent, as the race may be. Persons having known one fox-terrier will know much about all fox-terriers but would find a very different character and behavior for a pointer, a bull-dog, or any other breed.

The French bull-dog and the British bull-dog show marked achondroplasia associated with different degrees of dwarfism. The causal factors here are quite similar to those just mentioned. These animal types exhibit very characteristic reactions which distinguish them more or less from the ordinary dog breeds, such as pointers and setters, in a great number of ways.

The point of particular interest to us is that the type peculiarities exhibited by these dogs are very closely comparable to the peculiarities found in human individuals of these same general types.

Among the dogs, gigantism is shown by the Great Dane, St. Bernard, Newfoundland, and others, and here, as in the human being, gigantism is very commonly associated with acromegalia. It would seem both in dogs and man that dwarfism and achondroplasia like gigantism and acromegalia are usual combinations. Here again among these giant dogs, such as the St. Bernard, one finds general characteristics and behavior closely suggesting that of the human acromegalic giant. The voice and disposition are actually strikingly similar.

A discussion of these extreme types might be carried still further, but it seems quite evident I believe from these few examples that the nature of the type or constitution in these cases is definitely associated with somewhat peculiar and modified growth conditions probably brought about in response to altered glandular reactions. The cause of this glandular alteration may have been primarily genetic. Here again one finds all gradations from these extreme glandular conditions back to the ordinary normal individual. If we examine more carefully the so-called normal individuals, good observers may frequently note slight indications of the conditions considered above. The structural expression of individuals oscillates between certain delicately balanced growth tendencies which are greatly influenced by the conditions under which the individual develops.

In studying both embryonic development and postnatal growth I have been led to believe that the rate at which growth or development

occurs is a most important factor in determining the type and quality of the resulting structure. An individual that grows and differentiates rapidly, in the end shows a slightly different pattern from another which grows and differentiates more slowly. The rate of growth is no doubt dependent upon the rate of metabolism or rate of oxidation in the particular individual and, as far as our present knowledge goes, the one organ in the body having most to do in the long run with the rate of metabolism and growth is the thyroid gland. It may be that the slight differences in the activity of this gland have more or less to do with differentiating individuals into constitutional types.

When we study human beings as a group we find after careful inspection and on the basis of extensive anthropomorphic data that there tends to be in general two quite clearly contrasted types. The literature of anthropology indicates with surprising repetition that almost all anthropologists have originally recognized two human types. Roughly speaking, these are the long heads and the round heads. It is also true that when anthropologists have gone further with their studies they have become confused and have recognized in addition to the two original types a great number of other so-called types. When we look at the situation from a simple biological and developmental standpoint it seems easier to consider all the intermediate types as hybrids or mixtures of the two contrasted groups. A few years ago I attempted to point out the various contrasted characters in the two types and suggested that these simple differences in growth and development might result from slightly different metabolic rates in the two groups. This difference in rates of development was further associated with the thyroid activity of the individuals and this, in turn, might be decidedly influenced and affected by the nature of the environments in which the persons chanced to live for many generations. In general, it seems that all old and more or less sedentary races residing near the coast lines tend in type toward what is designated as the linear or high thyroid type, while the midcontinental people far removed from the sea and the source of iodine tended in the majority of cases to show a more rounded condition, a physiologically low thyroid or lateral type.

Since so many investigators in different fields have recognized these two types among human beings it seems strongly probable that such individuals actually exist and are typically differentiated from one

another. The differences between the two types are not only shown in many measureable morphological characters, but are equally evident in their physiological reactions and certain psychological tendencies. These types have also been recognized by psychiatrists, who have designated certain distinct differences between them, as introverts and extroverts, schizophrenic and cyclothymic and by numerous other terms.

The question of immediate connection and interest is whether, if these types do exist, they show different tendencies or diatheses towards the different diseases. Various clinicians from time to time have attempted to show that there are definite disease tendencies associated with certain type differences. Much of this work has been quite unsatisfactory, being very largely intuitive and impressionistic in nature. There is very little actually good quantitative data for consideration.

Recently, Dr. George Draper in New York has been conducting a study of various disease group individuals. He has not arranged the patients according to what might be considered type but has taken all individuals that actually showed a given disease and grouped these together. The measurements of the several groups have been compared. Many of his results are very important and are of considerable interest. The group comparisons often lend themselves, however, to further and different interpretations from those which Dr. Draper has pointed out. From this study of six different diseases, his curves and figures seem to indicate that only two of the disease groups are probably composed of a definite human type. The other groups are quite mixed in their characters and are probably composed of what one might term type crosses or hybrids. These disease groups differ from one another in more or less irregular ways and are not uniformly contrasted. It must always be recognized that a certain constitutional conditon may show the diathesis for a particular disease entirely aside from the actual type of the individual. Draper makes no attempt to check against this fact. We really have few facts to indicate that the constitution as such is evidenced in a definite way by particular physical measurements. A suprarenal depression, for example, may considerably modify constitution without altering physical measurements. Such conditions must be recognized by morphological appearance and the peculiar physiological reactions.

Draper's group that suffered from gall-bladder disease seems from the measurements to be composed largely of lateral type individuals, and the contrasted group exhibiting gastric or duodenal ulcer seems to be quite uniformly composed of linear type persons.

Members of these two disease groups are quite consistently contrasted with one another throughout the various measurements and indices which Dr. Draper has employed. Of course, it is not certain that all of his measurements or indices actually have any value or significance for type distinction since such a value for many of them has not been established. Certain physical proportions might readily be common to the two types just as hair and eye color are. It would not be expected, therefore, that these two groups should contrast in every way in which Draper has compared them, but certainly in many ways, and in all of those thought to be the most reliable qualities for type distinction they are definitely contrasted. Even to fine detail, such as toothpattern and hand and nail shapes, the ulcer group and the gall-bladder group exhibit the characteristics which have been pointed out by various investigators for the linear type and the lateral type respectively.

Sex of course is one of the most important factors in influencing both the constitution and the type of the individual, and very probably sex plays an important part in disease tendencies. Sex differences in the inheritance of haemophilia and other diseases indicates the importance in constitution of sex composition. As I have recently pointed out, the significance of sex constitution is also strikingly shown by the direction of the morphological changes which follow the removal of gonads in mammals and birds. Among the mammals where the female is believed to be homozygous for sex, having in her cells two X chromosomes, and the male heterozygote having only one X, the castrated or spayed individual as the case may be approaches in its general structural trend the female or homozygous sex. While among the birds with the male instead of the female homozygous for sex, the castrated and spayed individuals approach in their structural changes the general male appearance instead of the female.

It is interesting to find that in Draper's ulcer group which is composed largely of the typical linear individual, the female is very rarely included. This disease would seem to be in the large majority con-

fined to males. The linear type is rarely so fully developed or so well expressed by the female as by the male. In brother-sister groups, for example, the cephalic index is almost invariably higher among the females than among the males, and the female is more often rounded or lateral in her general structure than is the same class male. If gastric ulcer is a linear type disease then females might rarely show it as they are not usually of the pronounced linear type. On the other hand, it was found that in the gall-bladder disease group, the females predominated and the males of this group were rounded, fat, avirile individuals. Here again the round type is more fully expressed by the female than by the male in a mixed population. The general metabolism of the female is probably lower than for the male, as is indicated by her structural condition and reaction, although the difference may not be of such a magnitude as to be momentarily measured by the calorimeter.

The only other quite typical disease group shown by Draper's measurements was a small number of acromegalics. These of course are readily differentiated by ordinary morphologic criteria. The pernicious anemia group tended in their measurements very decidedly to approach the acromegalics. They had very large subcostal angles and had an average profile that tended toward the acromegalic picture. These anemic persons may by some possibility be a peculiar deviation from the bone growth state of the acromegalics. Both conditions may have some connection with calcium metabolism, and blood disturbance and bone disturbance in this way may be somehow interrelated.

It is most difficult at the present time to give any clear-cut or definite treatment of the relationship between human constitution and certain disease conditions. However, our present knowledge of inheritance and development along with a clearer understanding of the actions of the internal secretions in affecting body growth and form give present investigators a great advantage over earlier workers in understanding the relation of constitution to disease. The fundamental thing in this problem is in the first place clearly to determine the controlling elements in the development and differentiation of human constitutions. Until we actually understand the causes on account of which one constitution differs from another, it is most difficult to understand what their actual disease reactions mean. If there is any

direct connection between the constitution or the type and certain disease inclinations it then becomes highly important to devise more clean-cut scientific methods for distinguishing type. Type cannot be designated intuitively with actual reliability and there must be established some quantitative basis on which it may be clearly diagnosed. We must determine which are the important characteristics and features to be measured, remembering that soft parts may frequently be as characteristic as bony structures though not so easy to study and hence their neglect at the present time by anthropologists in general.

The entire subject of constitution, therefore, is one urgently demanding study and investigation. An initial appreciation of extreme types and large deviations from the normal along with the way in which these function, and react to disease, will give a more scientific appreciation of minor deviations and the normal types themselves. Such knowledge will be of value in both preventive and curative medicine. This need not in any sense mean that persons able to distinguish types may further predict the kind of diseases that a given individual will some day develop. The hope is rather more in the direction that when type is clearly known we shall be better able to understand how a patient will react to a particular disease.

An appreciation and understanding of the patient is certainly one of the greatest advantages the physician can hold in his effort to control and alleviate disease. The most important element in the complex situation of disease is the actual human individual involved and all effort and study towards an understanding of this element will scarcely be energy misdirected.

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CEREBRAL BIRTH INJURIES AND THEIR RESULTS

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From the Medical Clinic, Johns Hopkins Medical School, with the aid of a grant from the Epilepsy Medical Research Fund

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I. Introduction

A very extensive literature is to be found on the subject of birth injury of the central nervous system. Even in the last few years a number of important contributions have been made to the understanding of this important problem. The various types of intracranial lesions which may occur in the passage of the infant through the birth canal have been described with a detail and accuracy which leaves little to be desired and a very good understanding of the causes which lead to their production has been attained. On these pathological and obstetrical phases of the subject there is little to be said, because a very substantial agreement has been reached by most authorities. On the other hand, when we turn to the consideration of the late results of such birth injuries we find little exact information and the greatest difference of opinion. All types of congenital paralyses, athetosis, epilepsy, amentia and even hydrocephalus have been attributed by various authors to birth trauma often without the slightest evidence. It is the purpose of this paper to attempt a more exact definition of the group of true cerebral birth injuries. This work is the outcome of some unpublished experimental investigations which the writer has performed in association with Dr. Charles Bagley.

The material on which the following studies are based was obtained almost entirely from the records of the Harriet Lane Home for Children by the courtesy of Dr. John Howland. Case histories are also included from the records of the Surgical Department by permission of Dr. Walter Dandy, and from the Obstetrical Department by permission of Dr. J. Whitridge Williams. I am indebted to Dr. W. G. MacCallum for permission to include the pathological material.

The frequency and importance of birth injury is easily demonstrated by a few figures. One of the first important contributions to the morbid anatomy was made by Weyhe (1) in 1888. He published the autopsy findings of 959 still-born babies. Intracranial hemorrhage was found in 122 of these. Spencer (2) found hemorrhage in 40 per cent of his cases; Schott (3) in 30 per cent; Deluca (4) in 36 per cent; Schafer (5) in 20 per cent of 680 cases; Archibald (6) in 43 per cent; Warwick (7) in 43 per cent; Pierson (8) in 44 per cent; and Crothers

(9) in 65 per cent. Even higher percentages of intracranial hemorrhages are found in exceptional conditions such as breech delivery or prematurity. Ylppö (10) found injury of some degree in 90 per cent of premature infants. It is probable, however, that authors who find high percentages of intracranial hemorrhages include many small lesions which do not contribute to death. Holt and Howland (11) in summary of the figures obtained at the Sloane Hospital in New York state that about one-third of the deaths at birth, or in the first few days, are due to complications of labor.

There is some evidence that intracranial hemorrhage occurs in babies who survive and who may even show no clinical signs of birth injury. Weyhe, Doehle (12), Kundrat (13) and others have found old blood pigment in the meninges of children up to nine months of age who have given no evidence of birth injury at the time of birth. Recently Sharpe (14) has published the results of routine lumbar puncture in five series of 100 new-born babies each. In most cases the spinal fluid was removed in the first twenty-four or forty-eight hours. An average of 9 per cent of all new-born babies had bloody fluid. Very few of these showed any clinical signs of birth injury. Hines Roberts (15) has also performed lumbar puncture routinely on 423 colored babies and found blood in 60 fluids, or 14 per cent. Only 26 showed clinical signs of cerebral lesions and of these 12 died. It is obvious that these figures can not be accepted as conclusive, because it is not always possible to distinguish bleeding caused by the needle from fresh blood already in the spinal canal. It would seem preferable to defer lumbar puncture until the third or fourth day when any blood resulting from a birth injury would be tinged yellow and so easily recognizable. Nevertheless, these figures are not to be lightly disregarded as both authors have taken into consideration the causes of error and their figures are in substantial agreement. It is evident that intracerebral and subdural hemorrhages will not always reach the the spinal fluid. In fact, blood in the ventricles or the anterior subarachnoid space may not reach the spinal fluid at once.

In summary, the conclusion seems justified that a very large percentage of babies dying at birth, or within the first two weeks, show intracranial hemorrhage at autopsy. In only about one-third of the cases, however, is this hemorrhage extensive enough to cause death.

There is some evidence that a small percentage of new-born babies, who seem normal, or at most show mild signs of intracranial hemorrhage, have blood in the spinal fluid.

II. Pathological Anatomy of Cerebral Birth Injury

A. EARLY

A great variety of lesions has been ascribed by many different authors to birth injury. Weyhe described 122 cases of intracranial hemorrhage in the new-born. In 80 cases the bleeding was subdural; in 56 subarachnoid; intracerebral in 35; and intraventricular in 21. McNutt (16) found hemorrhages over the convexity in 8 cases and hemorrhage at the base in 2. Spencer reported bilateral hemorrhages over the convexity in 29 cases and unilateral in 20 cases. In 35 cases the hemorrhage was at the base and 7 times into the ventricles. Only 1 case showed hemorrhage into the substance of the brain. Warwick's cases showed bleeding over the convexity in 13 cases, bilateral in 7 and unilateral in 6; into the dura mater in 2; over the cerebellum in 1; and into the ventricles in 2 cases. In 2 cases of meningeal hemorrhage there was some softening under the clot. In no case was there any primary intracerebral bleeding. In summary she states, "All authors agree on the preponderance of bleeding over the cerebrum, usually limited to one side." Cushing (7) stated in 1905, "All extensive hemorrhages I have seen have been limited to one hemisphere and I am inclined to think the unilateral form is more common."

Holt and Howland's text book describes the findings as follows:

> The hemorrhages may be large or small. If small, they are frequently multiple and are found scattered over the convexity. . . . . Large hemorrhages may be at the base or at the convexity. . . . . Convexity hemorrhages are rarely limited to one hemisphere, although one side may be much more affected. It is usual for the blood to gravitate towards the base and become diffused. . . . . Hemorrhages between the dura and the skull may be said never to occur except when associated with fractures. . . . . In large hemorrhages the brain substance is softened and in places may be quite disintegrated. Hemorrhages into the membranes of the upper part of the cord are found in a large proportion of fatal cases.

Beneke (18) and several others have emphasized the frequency of

hemorrhage at the base and in the posterior fossa. Sietz (19) has claimed that the bleeding is at the base in half of all cases.

Schwartz (20) has found intracerebral hemorrhages and necrotic areas in a large percentage of children dying in the first six months of life. The most common lesions were petechial hemorrhages and minute necrotic areas in the central white matter and basal ganglia around the third and lateral ventricles. These are definitely related to the distribution of the tributaries of the vein of Galen; the vein lateralis ventriculi, vein choroids and the ventricular branch of the vein basalis. Such lesions are usually minute, but very extensive destruction of the central white matter and basal ganglia may result. In rare cases one or both hemispheres may be partially destroyed. He also describes several cases with cortical softenings which he relates to meningeal hemorrhages. In addition to these gross lesions Schwartz found evidence of a diffuse destruction of nerve tissue in a large percentage of infants. Brains which seemed normal grossly showed on microscopic examination numerous fat-laden phagocytes scattered throughout the brain as well as in small foci. Schwartz insists that the presence of phacocytes is always indicative of a pathological process and they are not to be found in new-born animals, whose brains are protected by solid skulls. Fisher (21) and Siegmund (22) have described similar lesions.

Extradural bleeding, always associated with fractured skull and usually with laceration of the brain, has been described by Archibald and Ballance (23).

Crothers (24) and Pierson have shown that injuries to the spinal cord and hemorrhage into the spinal canal with, or without, fracture of the spine are not uncommon especially in breech extraction.

Intraventricular hemorrhages were described by Ballantyne (25) and Eastman (26). Hemorrhages into the retina have been noted by Stumpf and Sicherer (27), Paul (28), and Jacobs (29) in from 20 to 50 per cent of all new-born babies. Voss (30), has been able to demonstrate bleeding into the inner ear in some cases.

In recent analysis of the autopsy findings in 80 cases of still-birth or neonatal death, Capon (31) gives the following sources of intracranial birth hemorrhage:

1. Small tentorial vessels coursing along the fibers of the tentorium may

be torn; the blood then collects in a film upon the upper surface of the cerebellum or upon the upper tentorial surface draining posteriorly around the occipital lobes.

2. Vena Magna Galeni, which is frequently distorted during the moulding of the head, may rupture at the point where the straight sinus is formed. The blood collects posterior to the mesencephalon and drains downwards around the cerebellum, pons and medulla.

3. Cerebral veins, near their terminations in the superior longitudinal sinus, may be injured. . . . . The effused blood flows downwards in the subdural space. Usually the hemorrhage is unilateral, but sometimes it is bilateral.

4. Superior longitudinal sinus, transverse sinus and straight sinus. It is only in the most severe types of birth-injury that such examples are found. The infant is almost invariably stillborn.

5. The internal cerebral veins, for instance the choroidal veins, may be damaged. These examples are rare, and are found almost exclusively in stillborn premature infants. The blood-collection may occupy the lateral, third and fourth ventricles; blood is also frequently found in these cases within the spinal membranes.

In summary we may say that in autopsies on the new-born large intracranial hemorrhages are frequently found. These are usually subdural, less often subarachnoid; and are situated with about equal frequency over the convexity and at the base. When the bleeding is over the convexity it is bilateral in about one-half the cases, but in most of these cases the amount of blood is unequal on two sides. Generally the blood diffuses throughout the subdural space and no softening of the cortex occurs, but local hematomas may occur and cause cortical softening. At the base large hemorrhages spread promptly throughout the meningeal spaces. Minute intracerebral hemorrhages are not uncommon in the central gray matter and brain-stem, and even large extravasations into the substance of the brain are sometimes found. Diffuse degenerations and focal necroses under the ependyma may be demonstrated in some cases. Fracture of the skull with extradural hematoma and fractured spines with injury to the spinal cord are not rare. Retinal and inner ear hemorrhages may be demonstrated

B. LATE

There does not seem to be any general agreement among neuropathologists about the late pathological anatomy of intracranial birth hemorrhages. A great variety of lesions has been ascribed to this cause without any proper evidence. Indeed, the same end result may be due to several initial processes so that it is often impossible to distinguish between prenatal, natal and postnatal disease. The most important problem is the outcome of the very common subdural, or subarachnoid, hemorrhage over the convexity. Buzzard and Greenfield (32) say vaguely, "Such extravasations (subdural) are, according to some observers, the cause of some cases of infantile hemiplegia or diplegia. . . . . The results of intracranial hemorrhage, whether subdural or subarachnoid in cases which survive . . . . are very similar. The coagulum is partly absorbed and partly organized so scar tissue, with or without the presence of cysts, forms the permanent remains" (11). Sachs (33) states that chronic "meningoencephalitis" sclerosis, cysts and partial atrophies are the late results of meningeal hemorrhages and Holt and Howland are in essential agreement with this view. It seems probable, however, that some of the cases which were formerly described as examples of "meningoencephalitis" are really properly classified as "atrophic lobar sclerosis" which is unrelated to birth injury.

In discussing the late results of meningeal hemorrhage it seems helpful to distinguish between diffuse hemorrhages in the subarachnoid and subdural spaces, and local or encapsulated hematomas. It is probable that hemorrhages which diffuse do little damage to the cortex and either cause death from increased intracranial pressure or are absorbed without leaving any lasting injury to the cortex. Blood extravasated into the subarachnoid space is rapidly absorbed and usually does not leave any marked changes in the leptomeninges. The writer has examined the brains of two patients who had suffered repeated intraventricular hemorrhages over a period of a year in one case and two months in the other. In both cases there was merely some yellow staining and a scarcely perceptible thickening of the pia-arachnoid. Yet there had been large amounts of blood in the spinal fluid. Symonds (34) mentions the case of a man who survived

subarachnoid hemorrhage and died of an intracerebral hemorrhage several months later. At necropsy no evidences of the first hemorrhage could be found. It is well known that any considerable amount of blood in the subdural space is organized and leaves a fibrous membrane under dura. This fact is supported by clinical experience with various types of "hemorrhagic pacchymeningitis" and by the results of experiment. Putnam and Cushing (35) state that when blood is injected experimentally under the dura it is organized from the dural surface and a fibrous, cystic membrane remains attached to the under surface of the dura. It has been pointed out in the preceding section that meningeal birth hemorrhages are usually in the subdural space and even in case the blood ruptures into the subarachnoid spaces the major part of the blood remains under the dura. It is evident, therefore, that we may expect to find evidence of old meningeal hemorrhage in the subdural space.

It is a common observation among surgeons that the brains of epileptics and diplegics show opacities of the pia-arachnoid and collections of fluid in between the sulci. Sharpe (36) states that this is the result of a meningeal hemorrhage at birth which has been partly organized. This appearance may be deceptive, however, and 2 cases are included in this paper which showed an apparent thickening of the pia-arachnoid at operation, but at autopsy the meninges were grossly and microscopically normal. It seems probable that this apparent thickening is really merely a meningeal edema, and not to be regarded as in any way the result of a birth injury.

Campbell (37), Tredgold (38), and Cushing have described localized cortical defects and scars which they attribute with reason to localized meningeal hemorrhages produced by birth trauma. Tredgold gives a good description of a lesion in the brain of an epileptic with right hemiplegia which dated from birth. The patient died at the age of thirty-three. At autopsy the lesion was confined to a triangular area over the left hemisphere with its base at the great longitudinal fissure and its apex at the anterior part of the Sylvian fissure. Over this area the pia-arachnoid was opaque and thickened and could not be removed without decortication. The underlying convolutions were somewhat distorted and atrophied. Microscopic examination showed a diminution of nerve cells in the affected areas and a secondary proliferation

of glial tissue. The opposite hemisphere was normal and no recent degeneration could be demonstrated by the Marchi method. In the cord the motor path from the left hemisphere was diminished and sclerotic. The dura mater was normal. Campbell writes as follows:

> In addition to the microgyrous condition affecting idiots, described in the foregoing section, there is one which is very common, and though more frequent in the parietal and occipital lobes than elsewhere, is not restricted to any particular part of the brain. The diseased area varies in extent and is represented by a small nucleus of puckered and contracted, or wholly destroyed gyri surrounded by others which are attenuated and sclerosed, but not deformed. There may be more than one lesion, and in any case considerable cerebral deformity and asymmetry results. . . . . I have seen many cases and the distribution of the lesions and the constant signs of postnecrotic destruction in the center of the area suggests a vascular lesion . . . . at birth or soon after.

Cushing shows an illustration of a small defect in one paracentral lobule causing paralysis of one leg. This he says resulted from birth injury.

The possibility that meningeal or intraventricular hemorrhage may result in organization and obstruction of the aqueduct of Sylvius or the subarachnoid spaces and so cause hydrocephalus has been suggested. The discussion of this problem will be reserved for a later chapter. Suffice it to say here that any large amount of blood in the meningeal spaces will invariably cause a severe reaction, increased intracranial pressure and, in infants, enlargement of the head.

One more condition must be mentioned among the possible late results of meningeal hemorrhage. Schwartz, Burham and Gerstenberger (39), Doehle and many older authors, have suggested that "hemorrhagic pacchymeningitis" of infancy might bear some relation to birth injury. This problem is a very intricate one and can not be adequately discussed here. However, Putnam and Cushing in their interesting paper on "Chronic Subdural Hematoma" have shown that in cases of traumatic subdural hemorrhage large endothelial lined spaces form under the dura and eventually become connected with the capillaries and filled with blood. These structures are very fragile and, it is believed, that they may rupture and cause the hematoma to

grow. It is possible that a slight injury to the head of a child with such a hematoma as a residuum of birth injury might result in further extravasation and the development of clinical symptoms. Burham and Gerstenberger believe that there are two factors involved, birth injury and a second injury later which may be slight. This view is not universally held and there is some evidence against it. It is merely mentioned for the sake of completeness.

The end result of the less frequent intracerebral hemorrhages is more clearly understood. We would expect to find intracerebral cavities containing yellow pigment usually asymmetrically situated and sometimes unilateral. A typical example of this type of lesion is described in Holt and Howland's Text Book.

Schwartz has been able to show that the periventricular hemorrhages and necroses which he has found in so many still-born infants result eventually, if the infant survives, in porencephalic cavities usually separated from the ventricular lumen by a thin membrane and crossed by trabeculae. Such cavities are often bilateral and nearly symmetrical and are usually considered examples of developmental defect. Schwartz, however, shows convincing illustrations of all stages in their development. He also shows beautiful examples of peripheral porencephalus resulting from cortical birth hemorrhage. Even the cases which show replacement of a whole hemisphere, or both hemispheres, by a thin walled sack, Schwartz believes, are due to birth injury. Fischer and Siegmund subscribe to these statements.

Two types of porencephaly, therefore, result from birth injury, the periventricular type produced by hemorrhage from the vein of Galen and the cortical type due to superficial injuries. These are properly examples of "false" porencephaly and it is customary to distinguish such cases from "true" porencephaly which is regarded as representing a defective development of the cerebral hemispheres. Various criteria are given for the distinction between the "true" and "false" types, but Collier (40) states that these are not sufficient and the differentiation is not always possible on anatomical grounds.

In summary, we would expect to find as the end result of former intracranial hemorrhage at birth the following conditions: Subdural hemorrhages might be expected to leave a fibrous membrane under the dura mater containing traces of old blood pigment. Diffuse sub-

arachnoid bleeding is probably absorbed without causing any permanent lesions except possibly some thickening of the meninges at the base. Encapsulated supracortical hematomas, it seems probable, result in local atrophy and sclerosis with thickening and possible cyst formation in the overlying meninges. These changes should be unilateral in at least half the cases and usually asymmetrical. Intracerebral cavities (false porencephaly) of two types are to be recognized; those resulting from cortical hemorrhage and containing old blood pigment in their walls and those, described by Schwartz, resulting from periventricular extravasations often bilateral and separated from the ventricle by a thin membrane. Some authors state that sometimes hydrocephalus and "hemorrhagic pacchymeningitis" are related to intracranial hemorrhage at birth.

III. Causes of Cerebral Birth Injury

The preëminence of trauma among the causes of intracranial hemorrhage in infants is now almost universally accepted. Obviously any condition which increases the stress which the foetal head sustains in its passage through the birth canal will increase the chances of injury. Contracted or malformed pelves, rigid soft parts, precipitate or prolonged labor, abnormal presentation, high forceps deliveries, breech extraction, or over-large foetal heads are all common factors which predispose to birth injury. These considerations are, however, outside the scope of this paper and will not be discussed further.

Illuminating analyses of the mechanical factors involved in birth injury have been published recently by Holland (41), Greenwood (42) and Ehrenfest (43).

The commonest type of hemorrhage which is found over the convexity in the subdural or, less frequently, the subarachnoid space is due to over-riding of the parietal bones and tearing of the tributary veins to the longitudinal sinus. Cushing has been able to demonstrate this lesion in two of his cases. He points out that these veins are very fragile in the infant and unsupported in the subdural space by Pacchionian granulations and connective tissue as in the adult. Moreover, they stretch between their fixed attachment to the sinus and the relatively movable brain. In some cases the longitudinal sinus may be torn and thrombosed.

Ehrenfest states that infrequently a similar over-lapping of the parietal bones over the occipital bone and tearing of the lateral sinus or the veins, which empty into it, may occur, more especially in cases of breech extraction.

Distortion of the head by excessive moulding is an even more important cause of dural tears. Beneke showed in 1910 that compression of the head in one diameter results in shortening of that diameter and a compensatory increase in some other diameter. Since pressure on the fetal head is usually antero-posterior or lateral or both, it is evident that the vertical diameter must be increased. This change in configuration is resisted by the dural ligaments, the falx and the tentorium, which are stretched and if the tension is great enough may be ruptured. Tears in the tentorium are very common and are most frequent near the insertion of the falx. Often the straight sinus or some of the larger veins are torn and a fatal hemorrhage at the base or over the cerebellum occurs. If the antero-posterior diameter is increased too much, the falx may tear, although this is not common. Beneke described 14 cases with tentorial tears which he had discovered in one hundred autopsies on still-born babies. He also published in detail his valuable method for the study of intracranial pathology. Beneke's work was soon confirmed by Sietz who found tentorial lacerations in half his cases. Recently Holland has given a lucid exposition of the forces concerned. He confirms and amplifies Beneke's observations. In his series of 167 autopsies, he demonstrated tentorial tears in 81 and laceration of the falx in addition in 5 cases. He showed that permanent asymmetry of the head, the so-called oblique head, may result from tears in the tentorium in cases that survive. Greenwood has since made exact studies of infants' heads by plaster casts taken immediately after birth and again a week later. He thinks that in normal vertex presentations the vertical diameter is not increased. On the contrary it may be shortened with over-lapping of the parietal bones. The antero-posterior diameter is, however, definitely lengthened. In brow presentations and breech extractions he has shown the vertical diameter is always increased.

Greenwood and Siegmund both think that the Schultze method of resuscitation may be a possible cause of dural tears.

In an excellent analysis of the intracranial pressure changes in

breech delivery, Crothers has shown that this method of delivery imposes a great strain on the tentorium. He points out that the force of uterine contraction and manual suprapubic pressure both tend to compress the walls of the supratentorial chamber and consequently cause pressure on the tentorium from above, and that traction on the body, which he shows is transmitted in part to the spinal cord, tends to draw the medulla down. The result is rupture of the tentorium and possibly herniation of the medulla into the foramen magnum with injury to the vital centers and death.

The cause of the multiple extravasations from the vein of Galen and its tributaries is believed by Schwartz to be stasis due to vasomotor disturbances. Holland gives a much better explanation. He points out that the vein of Galen extends between the fixed sinus and the relatively movable brain. In the process of moulding the vertex is raised and the vein of Galen is sharply kinked and stretched. This will occlude the vein and may even cause rupture of the vein or its tributaries. Holland has been able to demonstrate actual tears in the vein in several cases.

Another factor to be considered in all cases in which there is laceration of the large veins or dural sinuses is the interference with venous drainage which results. This must cause congestion and edema and add to the intracranial pressure. Dandy (44) has been able to produce a low grade of hydrocephalus by ligating the vein of Galen near its junction with the sinus. The increase in intracranial pressure resulting from lateral sinus thrombosis is well known to otologists.

Cerebral concussion and edema must result from the compression and moulding which the fetal head is subject to in difficult labors. Greenwoods' casts show almost incredible distortions. "Shearing stress" as well as direct compression is to be considered. Schwartz believes that the diffuse destructive changes which he has demonstrated are due to trauma and it is possible that such changes may be the explanation of those cases which show signs of intracranial injury and pressure without any bleeding to be found at autopsy.

Prolonged compression of the trunk during breech delivery causes intense intracranial congestion by forcing blood out of the venous reservoirs of the chest and abdomen back through the incompetent valves of the jugular veins into the head and neck. In adults severe

compression of the chest has been known to cause the extraordinary picture of "traumatic asphyxia" (45) in which the most intense cyanosis of the head and neck may persist for several days. Convulsions may occur under these conditions.

Multiple petechial hemorrhages in the brain and meninges, especially if associated with hemorrhages on the serous surfaces, are probably caused by asphyxia. Similar findings are seen in adults dying of suffocative asphyxia without head injury, and there is no reason to doubt that asphyxia may produce the same lesions in infants. There does not seem to be any clinical evidence that any recoverable degree of asphyxia unassociated with hemorrhage may permanently injure the pyramidal cells and yet spare the more resistant cells of the medulla. Nevertheless, Pike (46) and others have shown by experimental interruption of the cerebral circulation in animals that the cells of the medullary centers resist anemia longer than the cells of the cortex. The restoration of the cerebral circulation causes severe convulsions and if the animal survives it may show abnormal behavior, blindness and paralysis. No gross lesions are found in the brains of these animals when they are killed after several weeks, but microscopically necrosis of the cortical motor cells and degeneration of the pyramidal tracts can be demonstrated. There is no doubt that asphyxia increases bleeding from torn vessels by causing congestion, but there is considerable doubt whether it may produce rupture of large veins and considerable hemorrhage, unaided by other factors.

An important contributing cause of intracranial hemorrhage is prematurity. Couvelaire (48) found 18 per cent of premature babies born by easy spontaneous labors showed hemorrhages. Ylppö found intracranial traumatic lesions in 90 per cent of all premature infants. Most of these were unimportant, however. Paul states that the greatest incidence of retinal hemorrhages occurs in the premature. One-fourth of Warwick's cases of intracranial hemorrhage were in immature infants. Browne (48) states that intracranial bleeding occurs 16 times more frequently in premature infants than in full term babies. Raiz (49) discovered fatal hemorrhage in 23 per cent of premature stillborn babies and in only 5 per cent of babies born at term or after eight months. Both Sharpe and Roberts agree that prematurity favors

intracranial hemorrhage. In explanation for these facts Ylppö has proved that the cutaneous vessels of premature babies are abnormally fragile. He applied suction to the skin and determined the pressure at which hemorrhage occurred. In mature babies a pressure of 520 mm. Hg was required; in premature infants only 150 mm. Hg pressure caused rupture of the veins. He states that 7 per cent of premature infants are either mentally defective or develop spastic paralysis. Ehrenfest points out that the thinness of the premature infant's skull is a factor of importance in predisposing to excessive moulding and intracranial injury, and Schwartz found that animals whose skulls are solid do not suffer birth injuries.

The importance of prolonged coagulation time, which may give rise to hemorrhagic disease of the new-born, was first pointed out by Green (50). This factor has been emphasized by Warwick, Foote (51), Kaiser (52), Cruikshank (53) and Rodda (54). Warwick found gross hemorrhages in the viscera in 8 of her 18 cases of intracranial hemorrhages, and Rodda states that there were multiple hemorrhages in 20 per cent of her cases. On the other hand some authors are inclined to attribute less importance to this factor. Holt and Howland quote figures from Ritter and Townsend whose cases of hemorrhagic disease all together total 240. In only 4 out of this number did intracranial hemorrhage occur. Sharpe and Roberts could find only 3 babies with prolonged coagulation time in their total number of 105 with bloody spinal fluid. There can be no doubt, however, that spontaneous bleeding may occur in the skull from this cause just as it occurs in the abdominal viscera, and a delay in coagulation time no doubt will prolong the slow oozing from a small torn vessel until a large hematoma has accumulated.

Syphilis and other diseases of the fetus probably play some rôle. Brissaud (55) and Dejerine (56) have stated that syphilis favors intracranial birth injury by causing prematurity. Weyhe believed that 23 of his 122 cases of intracranial hemorrhage showed signs of congenital syphilis. Hedren (57), however, found only 3 cases of syphilis in autopsies on 700 infants, 9 per cent of whom showed intracranial bleeding. Warwick did not find any signs of syphilis in her 18 cases. Sharpe and Roberts do not consider syphilis important.

Cases of intracranial hemorrhage occurring in utero before labor

are not unknown. Cotard (58), Sietz Gibb (59) and Osler (60) have described cases of this nature.

IV. Results of Cerebral Birth Injury

Signs of birth injury

The early signs of intracranial hemorrhage in infants are familiar to all obstetricians. Recent studies have been made by Cameron (61), Capon, Henkle (62) and Holland, but little of importance has been added to the descriptions of the older writers. If the hemorrhage is extensive, the child may be still-born. If alive, it may be deeply asphyxiated and difficult to resuscitate. The "blue" asphyxia is of little significance, and it has been estimated that this occurs to some extent in 90 per cent of all new-born babies. The so called "pallid asphyxia" is more serious. Crothers has shown that this is in reality vasomotor collapse due to medullary injury. If respiration is established, it may be noticed in the next few days that the child is sluggish and unresponsive; more rarely it may be restless. The cry is feeble and the child does not nurse. Respiration is slow and irregular and there is often some cyanosis. The pulse is usually slow, but may be feeble and rapid. If the bleeding is over the cortex, there may be local muscular twitching or general convulsions. Rigidity and retraction of the head are often seen. Unequal pupils, nystagmus, strabismus and even exophthalmos are described. The fontanels may bulge, but this is not always to be relied upon. Changes in the retina may be found. On lumbar puncture the spinal fluid is bloody and under increased pressure. It is not unusual to have the signs delayed until the fourth or fifth day, when the indications of increased intracranial pressure give evidence that an intracranial hemorrhage has been slowly accumulating.

It is well known that some intracranial hemorrhages give no obvious signs. Sharpe says that scarcely any of his 45 cases could have been diagnosed without lumbar puncture. Roberts noted clinical signs of birth injury in only 14 children out of the 48 that survived. I have already mentioned that Weyhe and Kundrat state that it is common to find evidence of old hemorrhages which have caused no clinical signs. Schule (63) refers to a baby who died suddenly at six weeks with a

large hemorrhage from a torn tentorium and Manton (64) gives the history of a similar case in which death occurred on the tenth day from an extensive hemorrhage coming from a tear in the longitudinal sinus. Neither of these babies had shown any evidence of birth injury.

It may be well to state here that all the signs of intracranial injury enumerated above may occur in cases in which no intracranial hemorrhage is found at autopsy. It is probable that the pathology in some of these cases is cerebral edema from cerebral concussion, or perhaps, congestion. Sharpe believes he can demonstrate increased intracranial pressure in these cases by the spinal manometer. I shall show later that children with congenitally defective brains may also show signs which simulate birth injury.

The overwhelming majority of babies who show definite clinical signs of intracranial hemorrhage die within a few days. In one series which I have personally investigated only 7 out of a series of 50 in which the diagnosis was made survived for two weeks. It is evident that lesions of the neuraxis between the tentorium above and the phrenic nucleus below will be fatal in almost every instance. Lacerations of the large veins and the dural sinuses are probably almost always incompatible with life. Moderate supracortical hemorrhages probably have the lowest mortality.

PHYSIOLOGY OF THE INFANTILE NERVOUS SYSTEM

This peculiar lack of signs associated with intracranial hemorrhage in the new-born will be more comprehensible if we consider the physiology of the infant's central nervous system. At term the cortex and its projection tracts are still partially unmedullated, and, hence, it is generally assumed, are not functional. The pyramidal tracts do not become completely medullated until somewhere between the ninth and twenty-fourth months of extrauterine life. The segmental apparatus of the brain stem and spinal cord is functional and probably already completely medullated at birth. There is evidence that medullation of the various "association" tracts in the cortex continues for many years after birth. Two babies born in the maternity wards of J. Whitridge Williams, who showed all the reactions of normal babies including crying, nursing and vigorous movements, were found on necropsy to have been born without the cerebral hemispheres.

This observation is, in fact, a very old one and is attributed by Little to Ollivier. It indicates very strongly that the cortex plays no part in the normal activities of new-born babies. Pulse, respiratory rate, and temperature control are not well established at term. Tendon reflexes and the plantar reflexes are variable at birth and have no value in diagnosis as Burr (65) has shown. It would seem, therefore, that the normal activities of the new-born infant are all reflexes mediated by the brain stem and the spinal cord. However, the convulsions and local twitchings which sometimes result from cortical injuries, suggest that the cortex is not entirely insensitive to irritation.

Thus, it seems probable that the common signs of intracranial hemorrhage in infants result from involvement of the brain stem, either directly by bleeding into the posterior fossa, or indirectly by increase of intracranial pressure from the accumulation of a large hemorrhage above the tentorium. A localized hematoma over the cortex may compress and soften the cortex without giving any signs of its presence except, perhaps, some local twitching of convulsions. Respiratory disturbances without other evidence of intracranial injury have been attributed by Still (66) to small hemorrhages in the medulla, and Kirkwood and Meyer (67) have been able to demonstrate a small medullary hemorrhage in such a case.

LATE RESULTS OF INTRACRANIAL BIRTH HEMORRHAGE

It is quite certain that some few infants survive moderately large intracranial hemorrhages and afterwards show no clinical signs of cerebral injury. Numerous articles may be found in the literature advocating some type of operative procedure, subdural puncture or spinal drainage, and always including one or two histories of children who have survived intracranial hemorrhages and, strange to say, have not developed "Little's disease" afterwards. In each case this remarkable outcome is attributed only to the special treatment advocated by the author. Cushing (68) operated upon 9 babies with intracranial birth hemorrhage and stated in 1908, that the 4 who survived were all well. Most of these children had been under observation for over a year at that time. Gilles (69) reported 2 cases with complete recovery in children who were not subjected to operation, but were treated by lumbar puncture. Simmons (70) operated on a

child with intracranial hemorrhage who was quite well at the age of thirteen months. Green (71) reports 2 complete recoveries, one with operation and one with lumbar puncture. Lippman (72) described a case of meningeal hemorrhage in which only one lumbar puncture was performed. Nevertheless, the child made a complete recovery without residuum and developed normally. Brady (73) published 2 similar cases treated by spinal puncture. These children were well at the ages of three and fourteen months. Foote has followed a child who had a meningeal birth hemorrhage for four years and another for six years. Both these children are quite well. They were not subjected to operation. Rodda found 2 children who had shown blood in the spinal fluid at birth perfectly normal at the ages of four and ten months. Vaglio (74) reports 1 case of a child treated by lumbar puncture who is normal at six months. Munro (75) has published case histories of 9 children who survived intracranial hemorrhage and were well at the ages of one, six, nine, one, one, eight, eleven, eight and ten months. Most of these children were subjected to two lumbar punctures and no craniotomies were performed on the children who survived. Roberts does not believe that any of the 48 babies in his babies in his series who showed blood in the spinal fluid are going to develop diplegia, although the period of observation is still too short to be conclusive. The writer has been able to find records of three children who survived intracranial hemorrhages at birth and were well at the ages of six months, one year and nine months, and two years (see appendix A). In confirmation of these statements it may be mentioned that it is unusual for subarachnoid hemorrhage in adults to result in local cortical lesions. The writer has observed eight cases of primary subarachnoid hemorrhage all of whom recovered without any sign or symptom of cortical damage. One of these patients eventually had a recurrence and came to autopsy. No lesion of the brain was found.

Since the publication of Little's famous article in 1862 many divers clinical syndromes have been attributed to the more severe birth injuries. Spastic diplegias, hemiplegias, monoplegias, congenital athetoses, epilepsy, idiocy, and hydrocephalus are the most important. Cerebral diplegia, most frequently called "Little's disease," is, however, considered the most typical example of the late results of birth injury. All these conditions will be discussed in the following sections.

V. Relation of Cerebral Birth Injury to Spastic Paralysis

CONGENITAL DIPLEGIA

This is the commonest type of congenital cerebral paralysis, occurring in the Harriet Lane Home records many times more frequently than congenital hemiplegia. It may be defined as a congenital condition of spastic weakness which affects the legs more than the arms or the legs alone. The most severe cases show general rigidity of the extremities and bulbar palsy. The mildest cases show only slight spasticity of the legs and a tendency to walk on the toes. These are, in fact, paraplegias. In hemiplegia and double hemiplegia it is the rule in infants, as well as in adults, for the arms to be more severely affected than the legs. These distinctions make the differentiation of diplegias and double hemiplegias possible in most cases. Diplegia is associated almost invariably with some degree of mental defect and not infrequently with epilepsy and microcephaly. Severe forms are evident at birth. Milder forms are not usually noticed before the age of six months. The tendency in some cases is towards limited improvement. If there are frequent convulsions the course may be slowly progressive. Choreiform, athetotic, flaccid and ataxic varieties are described, but are less frequent than the spastic type. It is evident that the postnatal progressive diplegias should be excluded from this group, although Collier has shown that they bear a fairly close relationship to some of the congenital diplegias. The distinction which Little made between rigidity and weakness has been supported by continental writers, but it is difficult, if not impossible, to maintain.

Historical

In 1862 Little's (76) paper entitled "On the Influence of Abnormal Parturition, Difficult Labors, Premature Birth, Asphyxia, Neonatorum, on the Mental and Physical Condition of the Child, Especially in Relation to Deformities," appeared in the Transactions of the Obstetrical Society of London. It is evident from the following extract of that article that Little believed that asphyxia was the most important factor in the production of diplegia. He says,

> Nearly twenty years ago, in a course of lectures published in the Lancet, and more fully in a Treatise on Deformities, published in 1853, I showed that

premature birth, difficult labors, mechanical injuries during parturition to head and neck, where life had been saved, following the act of birth were apt to be succeeded by a determinate affection of the limbs of the child, which I designated spastic rigidity of the limbs of new-born children, spastic rigidity from asphyxia neonatorum. . . . .

Little did not attribute diplegia to gross hemorrhage, although this view is often attributed to him.

McNutt (77) in 1885 was one of the first to ascribe diplegia definitely to meningeal hemorrhage. She described the anatomical findings in ten babies dying of birth injuries and some months afterwards, reported a case of diplegia which she believed had resulted from a meningeal hemorrhage at birth. McNutt's paper was presented before the American Neurological Society as a thesis for admission and attracted universal attention. Many neurologists accepted her views, preëminent among whom was Sir William Gowers (78). Marfan (79), Raymond (80), Taylor (81), Osler (82) and many others have since lent their support to her theory which is now held by most pediatricians and neurologists with certain notable exceptions. Recently Cameron and Osman (83) and Sachs (84) have declared their belief in the meningeal hemorrhage theory, but it must be admitted that they have failed to offer any convincing evidence to support their opinion.

Brissaud, in 1894, influenced by the high incidence of prematurity in diplegic infants, suggested that the determining factor was prematurity which interrupted the development of the pyramidal tracts. He excludes cases with mental defect and epilepsy. According to Brissaud, the pyramidal tract has just reached the medulla at the seventh month of fetal life and premature birth at that time will cause generalized rigidity. At the eighth month the pyramidal fibers have reached the thoracic region and arrest of growth will cause paraplegia. After birth the pyramidal fibers may still grow slowly, hence these cases tend to improve, even to recover. Marie (85), Dejerine, and Van Gehuchten (86) have subscribed to this view with only minor reservations.

Freud (87) stated in his elaborate monograph of 1897 that he was unable to find any evidence that diplegia was due to birth injury.

He believes that premature, precipitate and difficult labor and asphyxia are all the result of the same causes that produce the diplegia. He shows that cases of diplegia dating from birth sometimes occur in the same families in which post-natal progressive diplegias develop, and assumes that the causes are similar. He refers to cases reported by Freer (88), Krafft-Ebing (89), Newmark (90), and Pelitzaeus (91) in support of these statements. Freud distinguishes very clearly between diplegias and double hemiplegias. Hemiplegias and double hemiplegias result from gross lesions, often birth hemorrhages; diplegias are the result of degenerative processes.

In 1900, Collier published an exhaustive analysis of the cerebral diplegias, including both anatomical and clinical evidence. He subjects the meningeal hemorrhage theory to very pertinent criticism and concludes that the pathological process is that of a neuronic degeneration which is usually pre-natal and non-progressive, but may be post-natal and progressive. Some cases, he admits, may be due to congenital defect. With few exceptions his views are identical with those of Freud. More recently Collier (92) has again illuminated the subject with a vigorous statement of his original point of view. His arguments will be given in greater detail in the discussion of the pathology.

Thus, five principal theories have been advanced to account for cerebral diplegia; that it is due to asphyxia, to meningeal hemorrhage, to an arrest of development resulting from prematurity, to a degenerative process in utero, and to a congenitally defective development of the brain.

Discussion

The reasons for connecting birth trauma and diplegia are not hard to find and a great deal is to be said both for and against this theory. We have seen that birth injuries are extraordinarily frequent, even in easy and apparently normal labors. It has been shown that although a great variety of intracranial lesions may occur, in infants born dead or dying soon after birth, one of the commonest lesions is a hemorrhage over the convexity near the midline so situated as to compress the cortical leg center. Such extravasations may be bilateral. Hemorrhage here can not diffuse so rapidly as at the base, where the subarachnoid spaces are larger, but is more apt to form an encapsulated hematoma

and compress the cortex. It is an old observation that diplegic babies are commonly premature, and are often born by precipitate or prolonged labor; all conditions which we know predispose to intracranial hemorrhage. These infants are difficult to resuscitate, cry feebly, nurse poorly and, not infrequently, have convulsions; all of which are suggestive of birth injury. When it becomes evident, in a few months, that mental development is not progressing properly and that the child is diplegic, the conclusion that these symptoms are the result of birth injury is almost irresistible.

On closer scrutiny the evidence that diplegia is due to meningeal hemorrhage is not conclusive, and there are many facts that point to a pre-natal cause. I am not aware that any large series of cases with definite clinical signs of intracranial hemorrhage at the time of birth have been followed to their ultimate outcome. This, indeed, would be every difficult to do because almost all cases with severe signs die in a few days or weeks. I have been unable to find a single report of a case of true cerebral diplegia in which the occurrence of an intracranial hemorrhage at birth was established. The direct evidence is, therefore, unavailable. The fact that extensive cerebral lesions are found in infants that die promptly is no proof that lesions of similar magnitude exist in infants that survive. It is well known, moreover, that meningeal hemorrhage in adults usually does not injure the cortex. Even the demonstration of blood in the spinal fluid can not be accepted as always indicative of brain injury. If Sharpe and Roberts are correct in their estimate that about ten per cent of all new-born babies have blood in their spinal fluid, it is evident that in the vast majority of cases it does no harm. The frequent, but not invariable, occurrence of difficulty in establishment of respiration, feeble cry and poor nursing may just as well be attributed to a congenitally defective nervous system as to a birth hemorrhage. The same argument may be advanced to explain the convulsions. Congenital defects of the nervous system are not at all rare, and every obstetrician is familiar with the grosser malformations. Indeed, 25 per cent of all diplegics are microcephalic.

It does not seem probable that asphyxia alone without gross hemorrhage can be of much importance. Little himself says, "It is obvious that the great majority of apparently still-born infants, whose lives

are saved by the attendant accoucheur, recover unharmed from that condition." Manton states that he has been unable to connect asphyxia with paralysis or other abnormalities of the nervous system. Buchardt (93) in a series of ninety babies deeply asphyxiated found only one abnormal later. Beatus (94) at Breslau came to the same conclusion. Walter Hannes (95) has followed three series of 150 cases each. The first group consisted of babies who were deeply asphyxiated and delivered by forceps, the second series consisted of babies not asphyxiated but delivered by forceps and the third included only babies normal in all respects. After some years the largest percentage of abnormalities of the nervous system was found in the third, or normal group, i.e., 3.4 per cent. The first and second groups showed 3.2 per cent and 3.3 per cent abnormalities of the nervous system respectively.

Brissaud's theory that prematurity is the important cause in the production of diplegia can not account for more than a certain percentage of all cases because far more than 50 per cent of cases are born at term. Brissaud, indeed, restricted his group so as to exclude all cases which were not premature. We shall see in the discussion on the morbid anatomy of diplegia that there is some pathological evidence to support his theory, that there is an arrest of development of the pyramidal tracts. There is no doubt, however, that over 90 per cent of premature babies that survive complete the development of their nervous system in time, and it is well known that medullation continues long after birth under normal conditions.

In the present imperfect state of our pathological knowledge of this subject, it scarcely seems justified to minimize the distinction between the congenital diplegias which tend to improve and the post-natal progressive cases, although Freud and Collier have shown that transitional cases occur. However, it is impossible to explain all cases of diplegia on one basis and we shall see in the next section that several different pathological conditions underlie this clinical syndrome.

Pathology of cerebral diplegia

The cerebral lesions that have been found in cases of cerebral diplegia are not always the same, and a great difference of opinion exists about their significance. Such cases come to autopsy long after the

active process has ceased and the end results may give little information about the original causes. The subject is further complicated by the fact that many authors do not give any accurate clinical classification of their cases.

Osler collected 31 diplegias and paraplegias. In 17 of these the pathological anatomy was available. In 3 cases bilateral porencephalic cavities were found. In the remaining 14 cases diffuse atrophy and sclerosis was the only finding.

Freud stated in summary of the large number of cases he had collected that the pathology of cerebral diplegia was a degenerative process and distinguishable from the lesions which result from birth injuries. He pointed out that the gross lesions sometimes described in diplegias could not serve to explain the symptoms which were due to neuronic degeneration demonstrable with certainty only with the microscope. Collier demonstrated in 1900 that the typical pathological picture was a diffuse symmetrical atrophy of both hemispheres with destruction of nerve cells and proliferation of glial tissue. The meninges are unaffected. In early cases the gross atrophy and gliosis are less evident and the degenerative process in the neurons is more conspicuous. The convolutional pattern of the hemispheres, which is established by the seventh month of fetal life, is usually normal but not infrequently shows evidence of interference in development long before birth. Sometimes the pyramidal tracts are absent; sometimes they are degenerated. Porencephaly of various types is found, but Collier very plausibly argues that these are cases of double hemiplegia, not true diplegia. He shows that the famous case reported by McNutt can not be in any way related to meningeal hemorrhage. Indeed, Welch, who made the pathological examination, states clearly that the process originated in the deeper layers of the cortex and involved the superficial layers least of all. Collier's own cases were all progressive post-natal cases and the pathology in these cases can scarcely be considered directly applicable to the problem of the congenital cases. In support of these statements Collier refers to autopsies by McNutt, Marie, Jendrassik, Warda, Mya and Levi, Sachs, Cotard, Schultze, Anton, Money, Hennoch, Forster, Isambert, Bechterew, Russell, Robin, Friedman, Muratoff, Schmaus, Gee, Richardiere, Bournville and Brissaud, Dejerine and Sollier.

Campbell describes the pathological condition which Freud and Collier believe is the basis of cerebral diplegia under the title of "lobar agenesis with sclerosis and microgyria." Buzzard and Greenfield employ the term "atrophic sclerosis," which they say gives rise to some types of idiocy, diplegia and microcephaly. Their description is as follows:

> Macroscopically, the chief appearance is that of atrophy, either localized or affecting to a greater or less extent the whole brain substance. In slight cases the convolutions when stripped of their meninges appear normal, but examination with a lens reveals here and there depressions or scar-like puckerings. The surface may have a finely worm-eaten appearance. In some advanced cases the convolutions may be irregular, atrophied, firmer than normal, and unlike healthy brain substance. In extreme degrees of the process the convolution is represented by a thin leaf of fibrous substance, the so-called "parchment-like convolution." Sections of such areas show numerous small cavities in the gray matter. The parts of the mid- and hind-brain associated with the sclerosed areas fail to develop, and the cerebral peduncles are smaller on the affected side. Microscopically the condition is one of neuroglial overgrowth associated with degeneration of the neuron substance. The process appears to affect primarily the deeper layers of the cortex, and spread thence to the underlying white matter and to the superficial layers of the cortex.

The authors consider this condition a congenital abnormality due to some pre-natal morbid process.

Hammarberg's (96) classical paper contained the description of 9 brains of idiots with various degrees of spastic diplegia. In each case a congenitally defective brain was found. In 4 cases there was complete amentia and severe spastic paralysis. The brains showed only one layer of cortical cells much diminished in number. These cells resembled the cortical cells of the fetus of five months. In 2 cases with some degree of mental development the cortex showed the development of a normal child of about one year. One of these children was severly paralyzed and its motor cortex showed very few giant cells. The second child had much better motility and the giant cells were correspondingly more numerous. Three more brains of children with slight or moderate amentia were studied. Two of these 3 patients were diplegic and few giant cells were to be found in their motor

cortex. The third child was not spastic and numerous giant cells were demonstrable in the motor area. Greenfield and Buzzard say "Another type of developmental defect associated with cerebral diplegia is shown by those brains which present the features associated with the early months of fetal existence, and which do not show the division of the cerebral hemispheres into numerous convolutions." They give an illustration of a brain of this type.

Ganghofner (97) has reported three cases of congenital diplegia in which the brain was grossly normal in every respect. Microscopic examination revealed nothing abnormal, yet he believed that there was probably some decrease in the pyramidal fibers in the cord which he could not demonstrate. The Betz cells in the motor cortex were normal. Two valuable papers have been published by Spiller (98), one in 1898 and one in 1905. In all he describes 3 very instructive cases of congenital diplegia with autopsy. All these brains were normally convoluted and showed no gross lesions. One was under normal size. Microscopic examination showed normal Betz cells in the paracentral lobule in each case, but in 1 case they seemed somewhat diminished in number. The pyramidal tracts in the cord showed no degeneration, but in 2 cases the fibers seemed abnormally fine. In the third case the pyramidal fibers seemed entirely normal, and Spiller could only conjecture that they did not reach the lumbar cord in normal numbers. This could not be actually demonstrated, however. Rhein (99) of Philadelphia has also contributed cases similar in every way to those described by Spiller. The motor cortex was normal except for the absence of Betz cells. The pyramidal tracts in the cord were abnormally fine but no degeneration could be found. Biswanger (100), Gerlich (101), Otto (102) and Haushalter (103) and Collins have also described similar cases. Many of these brains in which no definite anatomical basis for the rigidity could be found were studied before the introduction of modern histological methods. In some cases it is possible that the symptoms depended on basal ganglia lesions which were overlooked or could not be demonstrated.

Batten (104), whose exhaustive knowledge of the neurological affections of childhood enabled him to speak with authority, stated that cerebral diplegia is the result of four different pathological conditions; "atrophic sclerosis," arrested development of the brain, intra-

uterine occlusion of the cerebral vessels, and various types of meningitis. It is evident that he used the term diplegia to include all bilateral paralyses of cerebral origin. "Atrophic sclerosis," Batten believed, is the most frequent of all types of diplegia.

We must admit that it is possible for symmetrically placed lesions over the convexity near the midline to simulate the clinical picture of cerebral diplegia exactly. No doubt, bilateral meningeal hemorrhages may cause such a syndrome in rare cases, although the writer has been unable to find a report of any case of true congenital diplegia in which this lesion was found. Another possible cause of diplegia is thrombosis of the longitudinal sinus. Sir William Gowers stated that he believed some diplegias were due to sinus thrombosis, although he had no anatomical evidence to support the theory. In 1915, Holmes and Sargent (105) reported some cases of gunshot wounds of the vertex which involved the longitudinal sinus and produced a cerebral paraplegia with less severe involvement of the arms. Cushing (106) believes that it is the laceration of the cortex and not occlusion of the sinus which causes the paraplegia in such cases. More recently, Wilson (107) has published a case of paralysis of the legs resulting from syphilitic thrombosis of the longitudinal sinus in an adult. The writer has included in appendix C the case of a child in which thrombosis of the longitudinal sinus occurred at birth or very soon afterwards and resulted in general rigidity. Had this child lived it is possible that a picture similar to congenital diplegia might have developed. A similar case is also abstracted in which recovery without residual symptoms occurred. However, the anatomical evidence lends no support to the possibility that either meningeal hemorrhage or thrombosis of the longitudinal sinus is a frequent cause of congenital diplegia. A certain percentage of the bilateral congenital paralyses are due to porencephalic cavities in both hemispheres Etiologically these cases are different from the true diplegias and it is often possible to distinguish them clinically, since lesions deep in the hemispheres produce bilateral hemiplegias and not true diplegias. It seems important to make the distinction between diplegias and double hemiplegias whenever it is possible to do so.

The pathological anatomical basis of congenital athetosis is described by the Vogts (108) under the term of "étât marbré." In

this condition the lesion is restricted to the caudate nucleus and the putamen and consists in scattered areas in which nerve cells are absent and seem to be replaced by a fine network of medullated nerve fibers. In Weigert myelin stain preparations the striatum has a mottled appearance, hence the name. In a recent article the Vogts state that they now regard this condition as the result of a pre-natal process.

Analysis of cases from the Harriet Lane Home for Children

Two hundred and thirty-five cases of bilateral spastic paralysis are to be found in the records of the Harriet Lane Home. The writer has analysed 200 of these. These children were seen in the out-patient department, usually in early infancy. In many cases it was impossible to obtain an accurate history because of language difficulty or ignorance of the parents, but on the average the histories were full and usually contained negative as well as positive statements about birth and early infancy. Almost without exception these children suffered from severe malnutrition and the neurological condition was often completely overshadowed by their metabolic disturbances. Detailed neurological examination was often impossible because of illness or early age. Many of these children died while still under observation, although few were followed beyond a few weeks; and the majority probably failed to reach the second year. Under such circumstances it is impossible to differentiate this group with more than approximate accuracy, and, although it is certain that most of these cases are true diplegics and paraplegics, no doubt there are double hemiplegias included, and it is probable that a number of birth injuries are scattered through this group. A history of abnormal labor was obtained in only 15 per cent of cases. Convulsions at birth or in the first two weeks occurred in 16 per cent, and feeble respiration and nursing were noted in about 32 per cent. A history of prematurity was given in 33 per cent of cases. It is not wished to put too much stress on these figures because of their probable inaccuracy, but we will find in the analysis of the asymmetrical paralyses that a very much greater number of abnormalities of labor are reported. On examination, 71 or 35 per cent showed heads below normal size. Mental defect obviously could not be estimated accurately, but was almost invariably present. The most striking fact was the perfect bilateral

symmetry of the motor disturbance. In only 5 cases out of 200 was there any preponderance of paralysis on one side. This fact is hard to reconcile with the theory that such cases are due to birth injury, as we have found that in at least half the cases of hemorrhage over the convexity the extravasation is confined to one side, and when it is bilateral it is usually of unequal extent. Hence, we would expect, if birth injury were the true cause, to find just as many hemiplegias as diplegias and unequal involvement of the two sides in the diplegias. However, only seventeen congenital hemiplegias are to be found in the Harriet Lane records. It seems probable that most of the 5 cases in which the motor disturbance was asymmetrical were true birth palsies. However, the brain of one child with slightly unequal involvement of the two sides showed "atrophic sclerosis" at autopsy.

Autopsies on 6 diplegics[1] were secured, none of which revealed any condition to be attributed to birth injury. Two brains showed no gross abnormality. Only one was examined microscopically and no degenerations were found. These 2 cases, therefore, fall into the group described by Spiller. Three more brains showed the gross appearance of "atrophic lobar sclerosis," and one which was examined microscopically showed the characteristic appearance of that condition. The fifth brain was malformed, showing absence of the anterior corpus callosum, septum lucidum, fornix and falx.

It is interesting that Sachs and Osler found hemiplegias more frequent than diplegias, although many times as many diplegics as hemiplegics are included in the Harriet Lane records. It is probable that the tendency to nutritive disturbances and early death in the diplegic group may account for this discrepancy, as most of these children die before they reach the neurologist.

In conclusion we may say that true congenital cerebral diplegia is apparently unrelated to birth injury or meningeal hemorrhage. It depends on at least three anatomical conditions. Perhaps the commonest pathological picture is the so-called "atrophic lobar sclerosis" as described by Collier, Freud and others. The brains described by Spiller, Rhein, and Ganghofner which show slight defect in the pyramidal tracts undoubtedly constitute a distinct type of diplegia, which is

[1] A seventh necropsy has been secured recently. Grossly the brain is normal. Microscopic sections are not yet available.

probably to be regarded as a defect of development. The simply convoluted brains which indicate disturbances in development in the early months of fetal life are another type. The athetotic cases, the Vogts have shown, are dependent on the condition known as "état marbré." It is probable that spastic diplegia may be simulated by gross lesions including birth injuries, but it does not seem probable from the material available that any considerable percentage of cases can be accounted for in that way. Cases of birth injury are probably always to be distinguished from true diplegia anatomically, and the differentiation can usually be made clinically.

CONGENITAL MONOPLEGIAS, HEMIPLEGIAS AND DOUBLE HEMIPLEGIAS

In 1889, Osler wrote as follows,

> In infantile hemiplegia a great majority of the cases occur within the first three years of life, and in only a limited number is the condition congenital, either the result of intra-uterine disease or of accident during parturition. In bilateral hemiplegia and paraplegia the reverse holds good; in a large proportion of the cases the trouble dates from birth and is the result of injury to the child during its passage into the world.

Osler did not distinguish between double hemiplegias and diplegias, but his excellent description leaves no doubt that he was dealing with true diplegias.

Freud and Collier have taken a very different, almost exactly opposed, point of view. Collier concludes that monoplegia, hemiplegia, and even double hemiplegia may result from gross cerebral birth injury, but never true diplegia. He admits, however, that it may be impossible on clinical grounds to distinguish double hemiplegia from severe diplegia with general rigidity. The writer is entirely in sympathy with these views.

Batten believes that there are three pathological bases underlying congenital hemiplegias; unilateral arrest of development, intra-uterine occlusion of the cerebral vessels and birth injury to the cortex. The vascular lesions are the least frequent, he thinks. Unquestionably the great majority of infantile monoplegias and hemiplegias are acquired in the early years of life, as Osler states, but a considerable percentage of cases are congenital, and without doubt most of these

are the result of birth trauma. Indeed, Osler found 9 among his 120 infantile hemiplegias in which he believed such an etiology was probable. The writer discovered 17 congenital hemiplegias and monoplegias in a total number of 45 in the Harriet Lane Home records. These case histories are given in brief in appenxix C. In addition 8 congenital hemiplegias with focal convulsions were found in the records of the surgical department. In these cases operative notes are available. Brief abstracts are given in Appendix E. One case of congenital spastic paralysis of the leg is described in Appendix A. Eight cases of congenital spastic paralysis were found in a total of 200 cases of hydrocephalus. These are abstracted in Appendix D. The pathological anatomy was available in 10 cases in all. In 3 hemiplegias there was evidence of old organized subdural clot with atrophy and scarring of the cortex beneath. In 2 hemiplegias and 1 monoplegia there were found porencephalic cavities in the cortex. In 3 more hemiplegias there was local atrophy and scarring with thickening and cyst formation in the overlying pia-arachnoid. In 1 case of bilateral hemiplegia the periventricular type of porencephaly of Schwartz was found. In comparing these hemiplegias and monoplegias with the true diplegias the following facts seem significant. In these asymmetrical paralyses there is a history of abnormal labor in an extremely large (circa 70) percentage of all cases. The heads of these children are of normal size or even larger than normal, and sometimes asymmetrical. Intelligence is comparatively unaffected. The pathological findings are consistent with the diagnosis of birth injury.

Another group of cases may be considered with profit at this juncture. The condition known as "hemorrhagic pacchymeningitis," or infantile subdural hematoma, presents an interesting analogy to the meningeal hemorrhage which occurs at birth. The extravasation in "pacchymeningitis" is over the convexity and is often bilateral, in addition it is in some cases of traumatic origin. The clinical signs include papilledema with hemorrhages in the retina, enlargement of the head, vomiting, meningeal irritability and retraction of the head. There are usually no signs of focal cerebral lesions. The chief point of interest in the present discussion is that these children have large heads and not small ones, although most of these cases occur months after birth when the head does not enlarge so readily as in younger

infants. If a child with "pacchymeningitis" survives the acute stage of the malady, recovery may be complete. Rosenberg (109) reports that out of 14 patients he was able to trace after several years, 5 were dead of various illnesses, 2 were idiots, 1 an imbecile, 1 neurotic, 1 stuttered, 2 wet the bed and only 2 were quite normal. However, none were paralytic and no syndrome in any way resembling cerebral diplegia resulted. Only 1 of 6 cases observed by the writer showed any paralysis. In this case paralysis of one arm was noted.

From the material available the conclusion seems indicated that the congenital hemiplegias, monoplegias and the double hemiplegias, with large, normal or asymmetrical heads are in most cases to be considered true birth palsies. Intra-uterine arrests of development and vascular occlusions undoubtedly constitute a part of this group, but probably a smaller part.

VI. Relation of Cerebral Birth Injury to Hydrocephalus

It has been suggested by various authors from time to time that internal hydrocephalus might sometimes result from intracranial hemorrhage at birth. No very definite pathological evidence has been brought to support this theory, but it is quite conceivable that the organization of blood in the ventricular system or in the subarachnoid spaces might cause enough obstruction to the circulation of the cerebrospinal fluid to produce hydrocephalus. Siegmund and Crothers have mentioned hydrocephalus as one of the possible sequels of birth injury, and Ross (110) and Maclaire (111) have reported cases in which hydrocephalus developed in babies who had suffered birth injury. Fraser and Dott believe that some types of hydrocephalus are related to intracranial hemorrhage at birth.

CLINICAL DISCUSSION

Many congenital cases of hydrocephalus show little, or no enlargement of the head at birth. Often nothing is noticed until the child is two or three months old, when it is discovered that the head is increasing in size at an abnormal rate. Even at birth it is often possible to suspect hydrocephalus because of abnormally large fontanels, wide separation of the sutures, or thinness of the bones, especially when there is spina bifida or some other commonly associated malformation.

In such cases the diagnosis can be made definitely by ventriculography. If the progress is rapid the head may grow at an astonishing rate and there may be lethargy, vomiting and even convulsions. If the progress is slow there are usually few signs or symptoms and the atrophy of the brain is often extreme when the enlargement of the head is only moderate. Spastic weakness of the extremities and defective mental development are almost invariably present, but much less severe than one would expect from the reduction of brain tissue. Typically the optic nerves are pale and sharply defined, but there may be papilledema. Vision is generally somewhat reduced, and sometimes lost. Death may occur in a few months, or more rarely the process may become arrested and the patient may survive for years with a large head and more or less mental defect. Infrequently the head is much enlarged at birth so that delivery is difficult or impossible. Some of the cases of so-called "external hydrocephalus" are believed to be cases of internal hydrocephalus which have run their course in utero with collapse of the cortex. These cases reveal at section nothing but the basal ganglia and brain stem in a cranium full of clear fluid.

The hydrocephalus which so commonly follows meningitis usually develops more slowly than the congenital variety. After the cranial sutures unite firmly, which occurs at some time between the sixth and ninth month, enlargement of the head is much slower and no longer gives any information about the degree of cerebral reduction. After the fontanels are completely closed, which should occur before the second year, enlargement of the head is infrequent but may occur up to ten years in exceptional circumstances.

THE CIRCULATION OF THE CEREBROSPINAL FLUID

Much of our present knowledge of this subject is due to the fundamental work of Weed and Dandy. These authors have demonstrated that the cerebrospinal fluid is secreted almost entirely by the choroid plexus of the cerebral ventricles with the probability that a small part may originate from the perivascular spaces of the cortical vessels. This fluid formed in the lateral ventricles flows through the foramen of Monro into the third ventricle, through the aqueduct of Sylvius into the fourth ventricle and through the foramina of Luschka and the

median foramen of Magendie into the cisterna magna. From the cisterna the cerebrospinal fluid passes anteriorly under the base and then up over the convexity in the sulci between the cerebral convolutions where it is absorbed into the dural sinuses through the arachnoidal villi. After leaving the fourth ventricle the cerebrospinal fluid is confined entirely to the subarachnoid spaces.

PATHOLOGICAL ANATOMY OF INTERNAL HYDROCEPHALUS

No real understanding of this difficult subject existed prior to 1913, when the first of Dandy's brilliant contributions appeared, although a great deal of important but ill-correlated information had existed for many years. Even yet some authors fail to grasp the basic principles involved. Dandy has shown that almost all cases of hydrocephalus are due to obstruction at some point in the cerebrospinal fluid pathways. In some cases the obstruction is in the iter; in others at the foramina of exit of the fourth ventricle, and in a third type the block is in the cisterna at the base of the brain. The first two types are designated clinically as "non-communicating" hydrocephalus, because dye injected into the ventricles is not recovered in the spinal fluid on lumbar puncture. The third type is termed "communicating" hydrocephalus because the dye injected into the ventricles can be found in a few minutes in the lumbar cul-de-sac. Spiller (115) has described a case of unilateral hydrocephalus resulting from cicatricial stenosis of the foramen of Monro.

Cases have been reported in which an increased production of cerebrospinal fluid seemed to be the cause of hydrocephalus. Thrombosis of the vein of Galen has been found and "hypertrophy of the choroid plexus" is another unusual finding. These conditions are excessively rare and are mentioned merely for the sake of completeness.

When the iter is occluded, examination of the cut surface of the midbrain shows no trace of a canal. Microscopically a few strands of ependymal epithelium can be found in most cases, sometimes in the form of a flattened tube. There may be several small channels and sometimes minute cysts lined with epithelium are found. Spiller was the first to give a good microscopic description of this condition. Schlaff and Gere (116) have reported the anatomical findings in 8 cases of hydrocephalus with closure of the iter. Three cases showed

small tumors; 2 cases showed inflammatory lesions, one of which was syphilitic; and 3 showed the condition previously described by Spiller. Guthrie found 8 cases in which occlusion of the iter followed meningitis. One of Dandy's cases showed a thin transparent diaphragm stretching across the aqueduct.

The author has examined the midbrain microscopically in 11 cases of hydrocephalus in which the iter was occluded. In 5 cases the condition was congenital as shown by the history and presence of other congenital malformations. In these, several small channels lined with epithelium were found, but none of them seemed to be continuous (fig. 1). One got the impression that the iter had never been properly formed in these cases. In 2 cases with a history of meningitis an entirely different picture was seen. Here the outlines of an aqueduct of normal size could be made out distinctly, but the lumen was occupied by a fibrous mass of glial tissue. Many small spaces lined by ependymal cells were to be seen around the margins of this plug. These represented the original ependymal lining of the iter. In 1 case the signs of an active inflammatory process were still present (fig. 2). In 2 more cases, which will be discussed more fully later, there was a history of birth injury. In these cases exactly the same plugging of the aqueduct was found as in the post-meningitic cases (fig. 3). Thus it seems possible to distinguish clearly between the congenital cases in which the aqueduct has been imperfectly formed and those in which an iter of normal size has been plugged up by some post-natal process.

Dandy describes two different conditions found at operation and autopsy in cases of obstruction of the foramina of the fourth ventricle. In one the fourth ventricle is represented by a greatly dilated cyst with a thin, transparent wall. The vermis is lacking and the cerebellar hemispheres are small and displaced downwards. There are no adhesions and no other signs of a previous meningitis. He believes this is a congenital defect of development, and refers to the work of Blake (118) and Heuser (119) who have shown that the foramina of the fourth ventricle develop by a gradual thinning of the ventricle wall during early embryonic life. In most cases, however, he finds dense adhesions and thickening of the meninges, obviously the result of meningitis. The same condition may be found in congenital cases as in those which follow meningitis in infancy.

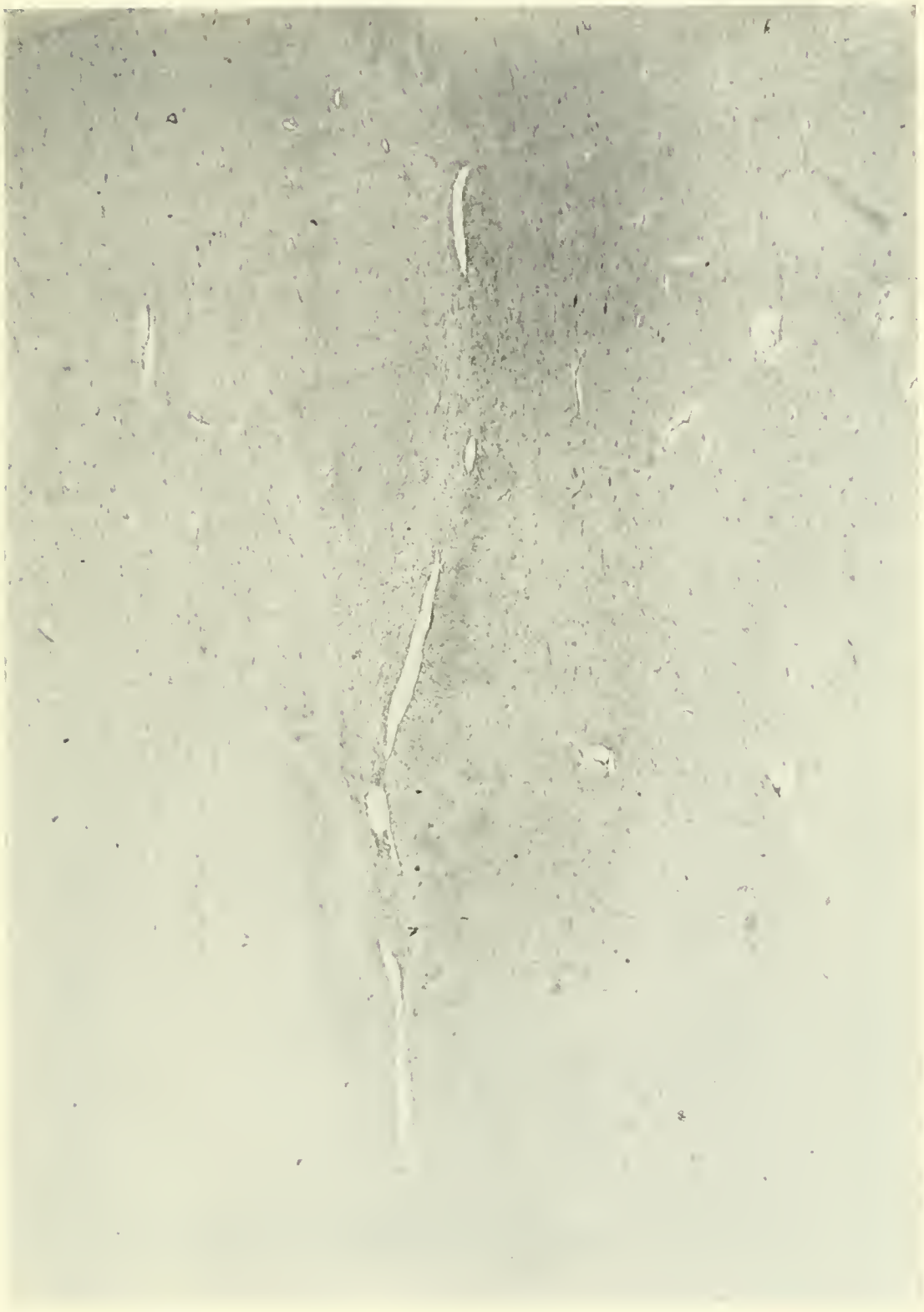

FIG. 1. SECTION OF CONGENITALLY MALFORMED AQUEDUCT SHOWING MULTIPLE INTERRUPTIONS OF NARROW SLIT

Fig. 2. Section of Well Formed Aqueduct "Plugged" by Organization of Inflammatory Exudate in Course of Meningitis

Evidences of inflammatory process still present

FIG. 3. SECTION OF WELL FORMED AQUEDUCT "PLUGGED" WITH FIBROUS TISSUE PROBABLY DUE TO ORGANIZATION OF AN OLD INTRAVENTRICULAR HEMORRHAGE AT BIRTH

In the communicating type, when the obstruction is at the base, there is in most cases evidence of old meningitis. Fraser and Dott have also invariably found adhesions at the base in this form of hydrocephalus which until Dandy's publications was called "idiopathic" hydrocephalus. Dandy also describes two cases of communicating hydrocephalus in which no signs of previous meningitis were found. He believes that these cases may be caused by a failure of the subarachnoid channels under the base to develop. Weed has shown that the subarachnoid spaces develop in pig embryos by a splitting of the periencephalic mesenchyme and begin to appear soon after the foramina have opened.

THE ETIOLOGY OF INTERNAL HYDROCEPHALUS

Dandy believes that, if we exclude the post-natal cases which are due to meningitis, hydrocephalus is always congenital. The fact that the head is of normal, or nearly normal, size at birth is not evidence against a congenital origin, because Dandy has been able to demonstrate by means of ventriculography that the brain is often greatly reduced at birth, although the head does not become noticeably enlarged until several months have passed. It is probable that the intra-uterine pressure has some effect in preventing the enlargement of the skull before birth. A small percentage of congenital cases, Dandy believes, are due to the failure of the foramina of the fourth ventricle or of the subarachnoid channels to develop. The vast majority are due to intra-uterine meningitis which may seal up the iter, the foramina of the fourth ventricle or obliterate the cisterna at the base. The evidence for this theory is that at operation, or autopsy, dense adhesions are found in the posterior fossa and under the base exactly like the adhesions which follow meningococcus meningitis in extra-uterine life. He has never been able to obtain any history of maternal illness which would give any explanation for this condition.

Fraser and Dott believe that intracranial hemorrhage is an important etiological factor which has been overlooked. "We possess very definite evidence that an intracranial hemorrhage produced at birth is liable to be followed by a hydrocephalus if the location of the hemorrhage is subtentorial in its distribution. These remarks apply only to ventricular (i.e., non-communicating) hydrocephalus." They ob-

tained a history of difficult delivery in 7 of their 21 cases and "subsequent operative interference revealed the presence of a hemorrhagic effusion in the membranes at the base of the brain." These conclusions are less convincing because the authors do not recognize congenital hydrocephalus unless there is spina bifida or the head is "considerably enlarged at birth, so much so, sometimes, as to constitute an obstruction to delivery." They admit, "In certain cases (without spina bifida) the statement was offered that the parents noticed the head to be somewhat enlarged at birth, but it is doubtful if any real weight can be attached to this observation." However, they have confirmed Dandy's statement that hydrocephalus may be demonstrated by ventriculography at birth in babies with heads of normal size.

On the basis of extensive pathological experience, Guthrie believes that post-natal cases of hydrocephalus are the result of meningitis and congenital cases are due to defects of development or intrauterine meningitis.

To determine whether birth injury has any importance in the causation of hydrocephalus, the writer has analyzed 100 autopsies of hydrocephalics (see Appendix D). In 11 cases there was evidence suggestive of intracranial birth hemorrhage, but 4 cases were at once excluded because there was definite proof that the hydrocephalus had existed at birth. In 2 other cases the head enlarged so rapidly after birth that it seems most probable that the condition was congenital. In 5 cases, however, it seems possible that the hydrocephalus may have been more closely connected with the intracranial hemorrhage. Two of these showed symptoms suggestive of birth injury in the first few days. They both had hemiplegia and later developed focal convulsions. Both had great reduction of the brain without enlargement of the skull. At autopsy both showed evidences of old subdural hemorrhage and destruction of the cortex with "plugging" of the aqueduct. It does not seem possible, however, to exclude with certainty an early inflammatory lesion. A third case was associated with hemorrhagic disease of the new-born and the ventricles were found to be small by ventricular puncture on the seventh day. Repeated hemorrhages occurred and the brain was greatly reduced at autopsy, but the skull was only moderately enlarged. The aqueduct was full of blood clot.

It is clear that the hydrocephalus in this case resulted from the bleeding, but is is not at all certain that a chronic hydrocephalus would have resulted if the child had lived longer. In 2 cases with histories of birth injury the periventricular cavities attributed by Schwartz to birth trauma were found. In both of these cases the aqueduct was occluded and in 1 case the description of the microscopic appearance of the aqueduct indicate that it was "plugged." In the other case the previous surgical dilatation of the aqueduct made it impossible to determine the type of obstruction.

An additional hundred cases of hydrocephalus from the records of the Harriet Lane Home for Children have also been analysed (see Appendix D). Autopsy was not obtained in these cases. In 5 cases there was evidence of intracranial hemorrhage at birth. However, the histories state that the head was noted to be abnormally large at birth in 4 of these cases, although in only 2 are exact measurements stated. In the other case no abnormality of the head was noted at birth, but it was impossible to obtain an accurate history so the diagnosis must remain at least doubtful.

Spontaneous subarachnoid or intraventricular hemorrhage in adults gives rise to marked signs of increased intracranial pressure and all the signs and symptoms of a meningeal reaction including some fever, leucocytosis in the blood and an increase in white cells in the cerebrospinal fluid. It is well known that the symptoms in such cases pass off when the blood is absorbed, and Symonds does not mention hydrocephalus as one of the sequels of subarachnoid hemorrhage in his excellent article on that subject. The writer has observed two patients in whom repeated intraventricular hemorrhages occurred over a series of several months. There was only a moderate dilatation of the ventricles at autopsy, and no organized obstruction in the ventricular system. These facts strongly indicate that intraventricular hemorrhage alone rarely, if ever, causes chronic hydrocephalus. Another factor must be considered. In five out of the six cases of hydrocephalus mentioned above which seemed to have some etiological relation to birth injury there was evidence of gross softenings of the brain tissue, and in four cases in which autopsy was performed it seemed probable that the lesion had extended down to the wall of the lateral ventricle. It seems possible that bits of necrotic brain tissue may be

carried into the aqueduct by the circulation of the cerebro-spinal fluid and remain long enough to give rise to an organized "plug."

In conclusion it seems evident that birth injury can not be more than an exceptional cause of chronic hydrocephalus. The extravasation of blood into the ventricular system or subarachnoid spaces of infants produces an increase in intracranial pressure, enlargement of the head, dilatation of the ventricles and a mild inflammatory process in the meninges. In the vast majority of cases such symptoms are transient and disappear when the blood is absorbed. It is possible that the aqueduct of Sylvius may be plugged by a blood clot or bits of necrotic brain tissue which becomes organized and a progressive hydrocephalus ensues. In six cases out of two hundred it is probable, but not certain, that this actually took place. Most of these cases were easily distinguished from the common type of chronic hydrocephalus. Almost all cases in which internal hydrocephalus and intracranial birth hemorrhage coexist are found to be cases of congenital hydrocephalus whose thin skulls and large heads have predisposed them to birth injury. It seems probable that some of the cases of arrested or non-progressive hydrocephalus associated with congenital hemiplegias and other spastic paralyses are to be attributed to intracranial injury at the time of birth.

VII. Relation of Epilepsy to Cerebral Birth Injury

"essential" epilepsy

By definition "essential" epilepsy bears no demonstrable relation to brain disease or injury, but occurs in otherwise healthy people without obvious cause. Clinically, however, the term is loosely used to apply to epilepsies without signs of focal brain lesion. It is well known that a certain percentage of such patients show gross brain lesions at necropsy. Southard (120) and Thom found only 68 grossly normal brains out of a total of 205 brains of epileptics. It is obviously difficult to obtain any figures of the incidence of birth injuries in such a clinical group because as soon as such an injury becomes probable the case is placed in another group. However, the recent studies of Patrick and Levy (121) throw some light on this problem. They compared the histories of 500 "ideopathic" epileptics with those of

750 unselected normal children. In only 5 of the epileptic cases was abnormal labor the "assigned" cause. The incidence of forceps delivery and asphyxia were about the same in both groups. In the epileptic group 3.6 per cent were delivered by forceps and 2.4 per cent were asphyxiated at birth. It must be borne in mind that these figures apply only to "essential" epilepsy in the clinical sense.

The anatomical changes found in "essential" epilepsy fall into three groups. First, developmental anomalies including various heterotopias and malformations of the cortical architecture. These findings have been stressed recently by Pollack (122) and by Geitlin (123). Secondly, acute changes in the nerve cells and fibers found in brains of patients dying in status epilepticus. Pierce Clark and Prout (124) gave a good description of these in 1903, and Bevan Lewis (125) demonstrated similar changes as early as 1890. Kogerer (126) has discussed the same problem recently. The third group includes the chronic changes such as degeneration and disappearance of the cortical cells, subpial gliosis and sclerosis of the cornu amonis. Spielmeyer (127) has given an exhaustive description of these lesions. Meyer (128) has shown, however, that the last two groups of changes are merely secondary and not to be considered in any sense characteristic. There is, therefore, no clinical or anatomical evidence to connect birth injury in any way with "essential" epilepsy.

"SECONDARY" EPILEPSY

The relation of recurrent convulsions to brain injuries of various types is established beyond any doubt. Fracture of the skull, gunshot wounds of the brain (129), cerebral neoplasms (130), granulomas, abscesses, scars of old inflammatory or vascular lesions, all are apt to result in convulsions, general or focal. Dandy (131) has found local areas of cortical atrophy and scarring at operation in a very large percentage of all cases of epilepsy. He has also been able to demonstrate these lesions by ventriculography since, as he shows, any loss of substance in the brain is compensated for by an out-pouching of the ventricle and an enlargement of the subarachnoid space. S. A. K. Wilson (132) has recently published a critical analysis of the relation of trauma to diseases of the nervous system. He writes as follows: "It has been asserted almost universally that trauma may cause

epilepsy; I have never been able to understand why. . . . . I associate myself entirely with Turner when he insists that 'it is difficult to avoid the conclusion that something more than local tissue alterations is requisite for the production of the seizures of traumatic epilepsy, and the determining agent, in my opinion, is an inherited or inborn constitutional predisposition to nervous instability and epilepsy.' R. G. Gordon has obtained evidence of a neuropathic predisposition in 75 per cent of his cases; in a series of my own, from the pensioners' clinic at the National Hospital, Queen Square, I have found similar evidence in 80 per cent." Obviously something more than the brain injury is necessary for the production of epilepsy because the vast majority of patients with cerebral lesions do not have convulsions, yet no one that deals with neurological patients and observes the frequent instances in which focal convulsions follow injuries to the brain can help feeling that brain injury is at least one factor in the production of epilepsy. It might be permissible to point out that "nervous instability" and "neuropathic predisposttion" are rather illdefined hereditary factors which most of us share with the epileptics.

In children, it is usually taught, brain injuries are even more likely to lead to epilepsy than in adults. Holt and Howland state that epilepsy occurs in from 33 to 50 per cent of all infantile cerebral palsies. Osler found 35 cases of epilepsy out of 120 infantile hemiplegias. Spratling states that 40 per cent of all infantile hemiplegias develop epilepsy. Five of the 17 cases of congenital hemiplegia from the Harriet Lane Home are subject to convulsions, and there is no doubt that about one-third of all children with spastic paralysis due to birth injury develop focal, or less frequently general convulsions. Eight cases of focal epilepsy associated with congenital hemipegias and monoplegias were found in the records of the surgical department. These cases are abstracted in Appendix E.

Spratling claims that 11 per cent of 1070 cases of epilepsy are to be attributed to infantile cerebral palsies. About one-third of these, he thinks, are congenital. In order to gain some idea of the frequency of "secondary" epilepsy following cerebral birth injury, the writer analyzed the histories of 100 unselected cases of epilepsy seen in the neurological out-patient department. In only two cases was there a history of birth palsy to be obtained, although there was a large percentage of

focal epilepsies in the group. However, it was felt that this result was inconclusive because many of the histories were meagre and probably inaccurate. Many of the patients were adults and had forgotten anything they might have been told about their birth.

Another series of 100 unselected case histories of epileptics from the Harriet Lane Home records was analyzed. Nine of these cases were of the focal, or Jacksonian, type and 3 of these were definitely associated with birth injury. Two of these 3 patients had congenital hemiplegias and the third had sustained an injury to the left side of the head at birth and later developed focal attacks on the right side. Five congenital diplegics were found in this group and 6 post-natal hemiplegias. In all 14 cases were associated with spastic paralyses. It is evident that these figures are in essential agreement with those of Spratling, and it seems probable that approximately 2 or 3 per cent of all epilepsies are related to birth injury.

VIII. Relation of Cerebral Birth Injury to Mental Defect

Imperfect mental development, or amentia, occurs in many different conditions, few of which are related in any way to abnormalities of labor. The overwhelming majority of such cases are due to congenital defects of various types. A common one is the "mongolian" idiot. The microcephalic with the simply convoluted brain is frequently encountered. Many microcephalics are also diplegic and have already been discussed. In cases of so-called simple amentia the brain may be grossly normal, but on microscopic examination a deficiency of cortical cells or an abnormal cortical architecture are revealed. Tuberose sclerosis is a rare type of congenital abnormality of the nervous system associated with multiple tumor formation and amentia. The "hypertrophic" brain is also a rare condition found in idiots with large heads. Such brains are of great size and weight and show extreme complexity of the convolutions. Scaphocephaly, oxycephaly and acrobradycephaly are all congenital malformations of the skull sometimes associated with mental defect. The most important factor in all these types of congenital defects of cerebral development, Mott believes, is a defective germ plasm. Tredgold and Goddard hold similar views.

Another group of conditions results in inhibition of mental develop-

ment soon after birth. Prominent among these is thyroid deficiency. Various types of infantile encephalitis, juvenile general paresis, and several forms of infantile cerebral degenerations belong in this group. Idiots by deprivation of sense organs, deaf-mutes, etc., may be related to early meningitis. Early epilepsies may also be included.

Only three types of mental defect have any possible relation to birth injury: the spastic, the hydrocephalic and the epileptic, all of which have been discussed already. There remains the remote possibility that some birth injuries might involve large areas of cortex without producing paralysis or epilepsy. Such cases must obviously be excessively rare.

Potts (134) has stated that 12 per cent of 5430 cases of mental defect are to be attributed to birth injuries. He apparently compiled these figures from the literature and gives no references. Beach and Shuttleworth (135) estimate that 17.5 per cent of idiots are the result of birth injuries. It is evident that these figures are excessive and it is probable that both authors have included the diplegias among the results of birth injury. Tredgold and Bolton (136), on the basis of an extensive pathological experience, both feel that mental defect of any pronounced degree is rarely related to birth injury. Tredgold states that not more than 1.5 per cent of all idiots can be traced to birth injury, and these are invariably epileptic.

IX. Conclusions

It is not possible to reach any final conclusions about the exact limits of the group of cerebral birth palsies from the evidence available. The cases of spastic paralysis have been difficult to analyse, and in many cases a satisfactory neurological examination has not been possible. Very few cases of intracranial hemorrhage which survived could be found and these have not been followed long enough. The pathological anatomy is very meager and usually imperfectly described and studied. Before any clear understanding of these problems can be secured a large series of detailed pathological-anatomical studies must be made with adequate clinical control. However, unsatisfactory as the material may be, certain impressions have been gained in the course of this investigation which it seems permissible to state in the form of tentative conclusions.

First, very convincing evidence has been gathered that the congenital diplegias which constitute by far the largest group of infantile spastic palsies as seen in the pediatrics department (235 out of 280 in all) are not to be attributed to meningeal hemorrhage at birth, but are the result of various pathological processes of intra-uterine origin. Four principle arguments support this conclusion. Meningeal hemorrhage is in at least half the cases unilateral, and when bilateral is almost always unequal on the two sides; cerebral diplegias are, with very few exceptions, bilaterally symmetrical. The heads of diplegics are usually either definitely microcephalic or slightly below normal size; meningeal hemorrhage causes rapid enlargement of the head. Children who are known to have intracranial bleeding at birth or in infancy do not develop cerebral diplegia. Lastly, the pathological anatomy of true congenital diplegia is such that it can not be reconciled with any theory of birth injury.

If the above statements are correct it will be seen that cerebral birth injuries are rare rather than common, and the great mass of infantile palsies can no longer be lightly attributed to faulty obstetrical procedures.

No final statement can be made about the relation of intracranial birth injury and chronic hydrocephalus. Undoubtedly, marked enlargement of the head may occur with extensive bleeding into the ventricular system or subarachnoid spaces, but in most cases the blood is eventually absorbed without leaving any permanent obstruction to the circulation of cerebrospinal fluid. Possibly in some cases chronic hydrocephalus may result from plugging of the aqueduct by a blood clot or a bit of necrotic brain tissue, and organization of the obstruction. It seems likely that some children with large heads and congenital asymmetrical spastic paralyses owe their condition to birth injury. Intracranial hemorrhage is not uncommonly an accidental complication of congenital hydrocephalus because these infants have large heads and thin skulls.

The common diffuse meningeal hemorrhage which is not large enough to cause death apparently leaves no residuum in the overwhelming majority of cases. The real birth injuries to the brain are caused by the rarer intracerebral hemorrhages and necroses, by depressed fractures with laceration of the brain, and undoubtedly, by

some more or less encapsulated meningeal hematomas which compress and soften the cortex. All the evidence which the writer has been able to collect seems to indicate that the true cerebral birth palsies are represented by the congenital hemiplegias, the monoplegias and the asymmetrical and unequal bilateral spastic paralyses. These children have large or normal heads, even in some cases asymmetrical heads. Their intelligence is on the average much greater than that of the diplegics. A history of birth injury may be obtained in a very large percentage of such cases and the pathological anatomy is quite consistent with the hypothesis of gross birth injury. Of course, it is realized that asymmetrical congenital defects of the brain are included with these cases and are probably clinically indistinguishable from them. Numerically this group, which the writer believes is principally composed of true cerebral birth palsies, is surprisingly small, just 17 out of 280 infantile cerebral palsies in the Harriet Lane Home records, if we exclude the 5 asymmetrical diplegias. These figures would mean that about 6 per cent of all infantile cerebral palsies are due to birth injury, but the group is not well enough defined to attempt any numerical estimation. Recurrent convulsions occur in about one-third or more of these cases, and we have already concluded that about 2or 3 per cent of all epilepsies seen in children are related to birth injury. Severe grades of mental defect are probably not related to birth injury with the exception of that type which develops in association with frequent convulsions. Tredgold estimates that not over 1.5 per cent of demented patients who die in asylums owe their condition to birth injury and it seems probable that these figures are very approximately correct.

I wish to thank Dr. Adolf Meyer under whose supervision this work was carried out, and Dr. John Howland, Dr. Walter E. Dandy and Dr. J. Whitridge Williams for their criticism and permission to use their case histories.

APPENDIX

A. Four Cases of Intracranial Birth Hemorrhage from the Obstetrical Department

There were approximately 50 cases in this series in which the diagnosis of intracranial birth hemorrhage was made or suspected. Out of the whole

group only 7 survived. In 3 cases lumbar puncture was not performed, and as no signs of an intracranial injury appeared later, these cases have been omitted. The remaining 4 cases are abstracted below.

Case 1 (Disp. H 38370, Obs. 10856)

M. C., first pregnancy, mother had seven convulsions, labor terminated by manual dilatation and high forceps, cut by forceps over left eye and left occiput, extraction difficult, deep asphyxia, did not cry for two hours, convulsions on second day, fontanels full, severe brachial plexus palsy on the left which improved gradually, at the age of several months mother noted child did not use right leg well, right arm also slightly clumsy, eyes always crossed, no more convulsions, mental development retarded, never learned to talk well.

P. E. At age of five years head 51 cm. in circumference, residuum of old upper arm brachial plexus palsy on the left, right leg very spastic and shows shortening and equinus position, left leg is used well, right arm is very slightly clumsy, eye movements are non-comitant, patient holds head to right as if there were right hemianopia, can say only a few words, but is good natured and obedient.

Case 2 (Obs. 10694, Ped. 26279)

L. B., colored, first child, delivered by mid forceps after manual dilatation, blades slipped and head was cut, deep asphyxia, resuscitated with difficulty, on second day fontanels bulged, marked rigidity, on third day spinal fluid showed much altered blood, rapid improvement and on discharge on sixteenth day seemed well.

P. E. At the age of two years head normal size, no weakness or paralysis, well nourished and mental condition seemed normal, moderate rickets.

Case 3 (Ped. 40512)

A. G., child of healthy parents, born at term by difficult labor, episiotomy was necessary, moderate cyanosis but seemed normal, on second day grew rigid and had many convulsions, spinal fluid full of slightly altered blood, jaundice, spinal fluid cleared up gradually in three weeks, three lumbar punctures done in all, on discharge at three weeks child seemed well.

P. E. At five months head 42 cm., well nourished and development seemed normal. At one year and nine months head 44.5 cm., no sign of spastic weakness, normal healthy child.

Case 4 (Obs. 15318)

Baby W., first child, premature rupture of membranes, perineal dystocia, low forceps, strong traction required, episiotomy necessary, moderate asphyxia, abrasion over each ear, on the second day rigid, restless, frequent general convulsions, fontanels tense, almost pure blood obtained from the cistern, on the third day fluid still bloody, rapid improvement, at thirteen days fluid almost clear, only slight rigidity.

P. E. At five months normal healthy child, no rigidity or weakness.

B. Analysis of 200 Cases of Congenital Bilateral Spastic Paralysis, Mostly Congenital Diplegias (from the Harriet Lane Home Records)

CONGENITAL CEREBRAL DIPLEGIA

Case 1 (Ped. 7040, Path. 5127)

M. L. McH., third child of healthy parents, one sibling living and well, second pregnancy resulted in four months miscarriage.

P. I. The patient was born prematurely at about seven months, labor was easy and no instruments were necessary, weight was 5 pounds, there was some asphyxia and the child nursed very poorly.

P. E. At seven days it was noted that the child was inert and signs of prematurity were evident. When seen again at three months there was some rigidity of the extremities, and a tendency to hold the legs crossed, the child swallowed with difficulty and was much undernourished. At eight months the head was 42 cm. in circumference. When child was handled there was intense muscular rigidity and opisthotonos; when undisturbed apparently no muscular spasm; when objects were placed in the child's hands they were grasped and held until they were removed; the optic nerve heads were normal; the tendon reflexes were increased. At three years the child could not talk but made grunting sounds and could express its wants by this method; it swallowed with difficulty; the head was held up but the child could not stand or walk without support, there was intense general spasticity and "scissors gait," tendon reflexes were increased, mental development seemed much retarded,—patient seemed to recognize parents, however.

Operation: The meninges were thought to be thickened and an excess of fluid in the subarachnoid space was found. Nothing more than an exploration was attempted. The child died soon afterwards.

Pathological anatomy: The brain and meninges seemed normal, the

vessels were normal, no adhesions were seen, the ventricles were not dilated, the convolutions were smooth and full, the sulci not widened. Microscopically the pyramidal tracts in the cord showed no degeneration, it seemed possible that there may have been fewer fibers than normal but one could not be sure of this, however, the fibers did seem somewhat finer and of irregular size as compared with the posterior column fibers. Sections of the paracentral lobule showed no degeneration or gliosis but large pyramidal cells were possibly diminished in number, and giant cells were not easy to find. Sections through the basal nuclei showed nothing abnormal. All the cell studies were difficult because the material stained poorly. The brain weighed 1250 G.

Case 2 (Ped. 33486, Path. 7978)

M. D., second child of healthy parents, the first child died in infancy from unknown cause.

P. I. Birth spontaneous at term, weight 7 pounds, did not cry, respiration very irregular, cyanotic at times, did not nurse.

P. E. When seen at three days head was 36.5 cm. in circumference, the fontanels were full but not bulging, there was general rigidity and frequent convulsions, respirations were irregular and gasping, the child could not nurse; after several days it was discharged improved. At seven months the head was 38 cm. respiration was now normal, but there was marked general rigidity, no mental development had occurred and there were frequent convulsions. Spinal fluid was normal. At nineteen months the head was 39.5 cm. in circumference, general rigidity and adductor spasm, marked under-nutrition, bronchopneumonia, death.

Pathological anatomy: The meninges were not thickened, the dural sinuses were patent, grossly there was some shrinking of the convolutions of the frontal lobes, the sulci in these regions were widened and possibly more numerous than normal, the shrinking seemed more marked at the foot of the gyri than at the apex, so some of the convolutions seemed undermined behind the central convolutions the cortex was grossly normal, the lateral ventricles were somewhat dilated, i.e., hydrocephalus ex vacuo.

Microscopic examination showed marked loss of nerve cells in the cortex of the frontal lobes and motor area and a secondary gliosis; there was no sign of an inflammatory process; in the spinal cord the pyramidal tracts were severely degenerated and there was also degeneration in the region of the uncrossed motor fibers and the direct and indirect cerebellar pathways (i.e., tracts of Gowers and Flechsig). The basal nuclei seemed normal.

Case 3 (Ped. 11112, Path. 4747)

T. H., first child of healthy parents, born by long labor, no forceps, jaundiced ten days, very ill, at five weeks was noted to be stiff.

TABLE 1

Analysis of 200 cases of congenital bilateral spastic paralysis

	NUMBER	PER CENT
Birth history:		
Convulsions in first two weeks	31	16.0
Feeble nursing and respiration	64	32.0
Difficult labor	30	15.0
Precipitate delivery	10	5.0
Prematurity	66	33.0
Heads:		
Microcephaly*	71	35.5
Oxycephaly	3	1.5
Scaphocephaly	1	0.5
Asymmetrical heads	3	1.5
Paralysis:		
Paraplegias	12	6.0
Spastic diplegias†	168	84.0
Asymmetrical bilateral paralyses‡	5	2.5
Athetotic diplegias	12	6.0
Choreic diplegias	1	0.5
Flaccid diplegias	2	1.0
Epilepsy:		
Convulsions, generalized	56	28.0
Pathological anatomy:		
"Atropic sclerosis"	3	
Gross malformation	1	
Grossly normal, defect in cortical cells (?)	2	
	6‡	

*This term should be qualified by the statement that the heads were usually only slightly under normal size and the children's general development almost always under par.

† It is possible that there are some bilateral hemiplegias in this group.

‡ Abstracted in appendix B.

P. E. At four and one-half months poorly nourished, head 35 cm. in circumference, internal squint, all tendon reflexes increased, cannot sit up or hold up his head, general spastic rigidity, spinal fluid negative, death of malnutrition and furunculosis.

Pathological anatomy: Brain showed full round convolutions, meninges

delicate, sulci not widened, no abnormalities of the vessels, ventricles not enlarged, microscopic examination not made, brain cannot be found for further examination.

Case 4 (Ped. 41534, Path. 8207)

W. J. Z., birth by easy spontaneous labor, no evidences of birth injury, nursed well and seemed normal until four months when it was noted that child did not hold up head normally, never could sit up, no signs of mental development, convulsions at six months, diarrhea and digestive disturbance developed.

P. E. At six months head 35.5 cm. in cirucmference, and somewhat pointed at top, weakness of all extremities, tendon reflexes increased, very little spasticity, no signs of mental development, marked under-nutrition, and death from pneumonia and otitis media.

Pathological anatomy: Brain of normal size, falx and longitudinal sinus extend anteriorly only to fissure of Rolando, septum lucidum, anterior part of corpus callosum and fornix are lacking, anterior to the falx the convolutions interlock but do not fuse, rest of the brain shows no gross abnormalities; no microscopic examination made.

Case 5 (Ped. 404445, Path. 8059)

W. H., born by easy, spontaneous labor at term, three sibs normal, mothers' first pregnancy was an eighth month miscarriage, patient had severe jaundice and many convulsions on the third day, very ill, never considered a normal baby, never learned to hold up head, or use arms or legs well, left arm was used a little better than the right, numerous "rigid spells" probably convulsions, no mental development.

P. E. At fourteen months head 48.5 cm. in circumference, somewhat flattened in biparietal diameter and increased anteroposteriorly, left side of forehead is less prominent than the right, marked adductor spasm and spasticity of both legs, arms are relatively spared, but there is some motor disturbance in both arms, more severe on the right, tendon reflexes increased, bilateral extensor response, no signs of mental development, death a few days later from otitis media and pneumonia.

Pathological anatomy: Gross atrophy of both frontal lobes, more severe on the left side, other lobes of brain are apparently normal and show full round convolutions, meninges delicate and vessels normal, gross appearance like that of brain in case 2. No microscopic examination made; brain cannot be found.

Case 6 (Ped. 39402, Path. 8302)

P. T., full term, normal delivery, duration six hours, no forceps, deeply asphyxiated, three hours before child could be resuscitated, weight seven and one-half pounds, convulsions after breathing started.

P. E. At two days head 36 cm., no superficial injuries, slight cyanosis, general rigidity noted, tendon reflexes increased, swallowing difficult, regurgitation through nose, fed by gavage, vomited, thought to have intracranial hemorrhage but cistern puncture gave normal fluid, convulsions ceased at six days. At five weeks head 38 cm., nutrition poor. At seven months head 43 cm., severe nutritive disturbance, furuncles, otitis media, pneumonia, death. Never showed any signs of mental development. always definitely rigid but neurological condition was overshadowed by metabolic disturbance.

Pathological anatomy: Brain small, weight 690 grams, atrophy of convolutions over whole surface but especially those on either side of the Sylvian fissure; brain abnormally hard, meninges normal, vessels normal, wasting perfectly symmetrical, spinal cord not examined, no signs of hemorrhage, no microscopic examination. Brain cannot be found.

ASYMMETRICAL AND UNEQUAL BILATERAL CONGENITAL SPASTIC PARALYSES

Case 7 (Ped. 27744)

E. E. B., born at term by long difficult labor, forceps required, deep cyanosis, difficult resuscitation, very limp and weak for a week; did not nurse. Face asymmetrical, drawn to the right, no convulsions, left arm and leg were never moved freely; gradual improvement, has always seemed bright.

P. E. At ten months head normal size, child seems normally alert, left arm very spastic, right arm used well, some motor disturbances in both legs, more marked on the left, back and neck also weak.

Case 8 (Ped. 36233)

V. M. C., first child, born at eight months by difficult forceps delivery through contracted pelvis—said to have been injured but exact details are not available,—difficulty in using extremities noted in first few months.

P. E. At three years head normal, right arm very spastic, both legs show some spasticity but this is more marked on the right, some tendency to internal strabismus; mental development seems normal.

Case 9 (Ped. 16611)

W. H., second pregnancy, first was a miscarriage, born at term by difficult breech delivery, no signs of injury noted. At three months it was noted that child did not use left arm and leg as well as the right, late in holding up head.

P. E. At two years head 50 cm. in circumference, seems normally intelligent, left arm spastic, some spasticity and adductor spasm in the legs not stated to be unequal.

Case 10 (Ped. 20710)

W. P. W., third pregnancy, first two were miscarriages. No history of birth available. At two months it was noted patient did not use left limbs well.

P. E. At three and one-half years head 53.3 cm.; left arm very spastic, and smaller than the right; both legs somewhat spastic, the left leg is shorter than the right and seems more severely involved, no note of intelligence, poor history, family are very ignorant.

Case 11 (Ped. 24313)

F. S., first child, labor stated to have been normal, but no details available. no birth injury noted, never used left arm or leg well.

P. E. At five years head normal size, left arm very spastic and smaller than the right, both legs slightly spastic, the left one worse, mental condition not noted.

All five of these cases showed essentially a well marked hemiplegia (with greater involvement of the arm than the leg) complicated by a spastic weakness of the opposite leg which was not so severely affected as the homolateral leg. It is unfortunate that no observations upon the pathological anatomy are available and that the histories were not very satisfactory. However, a history indicative of possible birth injury was secured in several cases and it seems probable that most of these cases are true birth palsies. A case of bilateral spasticity due to porencephalic cavities in both cerebral hemispheres is included in appendix D. The cavities are periventricular and very similar to the central porencephaly described by Schwartz.

C. Analysis of 17 Cases of Congenital Hemiplegia and Monoplegia from the Harriet Lane Home Records

Case 1 (Ped. 7708)

C. S. No accurate information about birth, said to have weighed 12 pounds, series of convulsions at six days, never opened left eye, vomited frequently.

P. E. At three weeks head was 40 cm., sutures were widely separated, left lid drooped, left pupil large, fixed, left eye turns out, weakness of left deltoid probably peripheral, head asymmetrical, left frontal region flattened, all tendon reflexes increased, general increase in muscular tone, much old

TABLE 2

Analysis of 17 cases of congenital monoplegia and hemiplegia

	NUMBER
*Birth history:**	
Convulsions in first two weeks	4
Feeble nursing and respiration	5
Difficult labor	9
Precipitate delivery	1
Prematurity	1
Heads:	
Microcephaly	0
Asymmetrical heads	4
Paralysis:	
Hemiplegias	16
Monoplegias	1
Epilepsy:	
Convulsions, focal	5

* No history of birth was secured in 2 cases.

blood in spinal fluid. At two months head was 41 cm. in circumference, spastic of the right arm and leg was noted, one general convulsion. At three months head was 41 cm. in circumference, no more convulsions, bright child, right sided weakness still evident. At five months mental development seemed satisfactory. Right arm and leg spastic, third nerve palsy all gone except for large pupil, head still asymmetrical.

Case 2 (Ped. 29893)

F. B., first child, instrumental delivery, did not breathe well, two large cephalhematomas, vomited blood for four days. At nine months it was

noted that right arm and leg were not used normally; leg has improved more than the arm.

P. E. At five years head normal size, moderately severe right hemiplegia, extremities on right are somewhat underdeveloped, mental condition not noted.

Case 3 (Ped. 17855)

L. L., third pregnancy, born by spontaneous labor, no history of dystocia, first two pregnancies resulted in miscarriages, child never used right arm or leg well; otherwise seems normal.

P. E. At one year, size of head not given, partial right hemiplegia, mental condition not noted, poor examination and history.

Case 4 (Ped. 35242)

A. O., first child, born at seven months by normal labor, never able to use right arm or leg well, mental development apparently normal, no convulsions noted.

P. E. At twenty months head large, symmetrical, spastic weakness of right arm and leg with some under-development, child seems mentally normal.

Case 5 (Ped. 38753)

E. D., third child, born by precipitate labor, very cyanotic, did not cry or nurse for three days; on the ninth day twitching on the left side of the face, left arm and leg were cold and blue from birth and patient never moved them much for six months; eyes were crossed; at four months focal convulsions started on the left side.

P. E. At seven years head 51 cm., in circumference, left arm and leg weak and spastic, right external rectus weak; mental condition not obviously subnormal.

Case 6 (Ped. 23418)

B. E., first child, born by long difficult labor, forceps extraction, head lacerated, occiput flattened, probable fracture of skull, convulsions for six days, never used right arm and leg well, mental development fairly good, passed at school, infrequent focal convulsions on right, starting at six years.

P. E. At eleven years head 53.5 cm. in circumference, speech difficult, moderately severe right hemiplegia; mental development nearly normal.

Case 7 (Ped. 16279)

M. A., third pregnancy, forceps delivery at term, labor lasted sixty hours, right ear and scalp cut, left facial paralysis apparently peripheral, always dragged right leg and never used right arm well.

P. E. At two years head normal size mental condition normal, slight residuum of right hemiplegia.

Case 8 (Ped. 4113)

G. H., full term labor terminated by forceps, did not cry for several days, did not nurse, when child was only a few months old it was noted that the left arm and leg were not used well.

P. E. At five years partial left hemiplegia with under-development intelligence normal, head normal size.

Case 9 (Ped. 8494)

I. K., first child born by difficult labor, forceps used, birth weight fourteen pounds, laceration of right side of head, convulsions on second day, weakness of left arm and leg noted at age of three months.

P. E. At two years head of normal size, old depressed fracture on right side, partial left hemiplegia, not much difference in size of extremities on two sides, mental development only slightly retarded.

Case 10 (Ped. 40459)

F. G., first child, born at term (?) by difficult high forceps extraction, birth weight 4 pounds, child never used right arm and leg well.

P. E. At twenty-two months head of normal size, no difference in size of limbs yet apparent, severe right hemiplegia, no convulsions noted, mental condition not noted.

Case 11 (Ped. 4787)

E. G., fifth child, born by full term normal labor, development retarded, did not walk until age of two years, talked at two years and three months, never used right arm and leg well.

P. E. At six years head 50 cm. in circumference; mental condition normal; severe right hemiplegia with underdevelopment of extremities on right.

Case 12 (Ped. 6577)

H. L., sixth child, full term, normal labor, weight 8 pounds; did not walk or talk until age of two years; weakness of right arm and leg noted at age of

eight months, probably congenital, focal convulsions started on right at age of two years.

P. E. At eight years, head 48 cm. in circumference, right arm and leg spastic and poorly developed; some mental defect—Binet Simon mental age of 7.4 years, internal strabismus.

Case 13 (Ped. 32123)

J. S., fourth pregnancy, twin died at seven months in convulsions, fifth pregnancy died during delivery, first three living and well, child born by normal labor at term; weakness of right arm and leg noted as soon as child began to use them.

P. E. At four years head of normal size mental condition normal. right arm a little spastic, both legs are used normally.

Case 14 (Ped. 38870)

C. Z., first child, born at seven months by difficult forceps extraction, poor history of birth, weakness of right arm and leg noted when child began to use them, left arm was thought to have been weak at first.

P. E. At two years head of normal size; partial right hemiplegia; no obvious difference in size of extremities on the two sides, mental development fairly good.

Case 15 (Ped. 43811)

G. C., first child, born by difficult forceps delivery, forehead cut, never used right arm or leg well, onset of focal convulsions at three months on the right side.

P. E. At eight months head 44.5 cm. in circumference, symmetrical, tendency to internal strabismus, spastic weakness of right arm and leg, face very slightly involved, no obvious difference in size of extremities on the two sides, mental development seems good.

Case 16 (Ped. 29454)

W. W. K., twelfth child of healthy parents, said to have been born by normal labor, never used right arm or leg very well, focal convulsions on the right side at long intervals.

P. E. At five years head 47.5 cm. in circumference, right arm and leg very spastic and smaller than extremities on the left; mental condition not noted.

Case 17 (Ped. 34876)

E. B., first child, full term forceps delivery; no other information about birth available, never used right arm or leg well.

P. E. At fifteen months head large, right side flattened, internal strabismus, right arm and leg are weak and spastic, and somewhat underdeveloped; seems bright.

TWO CASES OF THROMBOSIS OF THE SUPERIOR LONGITUDINAL SINUS

Case 18 (Ped. 42061, Path. 8294)

E. E., birth spontaneous at term, lasted eighteen hours, deep asphyxia, did not breathe, revived with difficulty, next day general twitching, vomited, limbs flaccid, very ill.

P. E. At twelve days flaccid paralysis of all limbs, becoming spastic in two days, general muscular twitching, fontanels flat, pupils small and fixed, internal squint, spinal fluid clear, longitudinal sinus not punctured; pneumonia and death at fifteen days.

Pathological anatomy: Thrombus in longitudinal sinus showing beginning organization, brain soft, not carefully examined, cannot be found for further examination.

Case 19 (Ped. 44555)

S. M., tenth child, sibs all living and well, born by long dry labor, no signs of birth injury; on second day developed ecchymoses over face and trunk, vomited blood; on the fifth day left arm began to twitch and later the right arm was involved.

P. E. At six days rhythmical twitching of all extremities, more evident in the left arm and leg; jaundice; fontanels sunken, no rigidity or definite paralysis, tendon reflexes all very active, no clonus; spinal fluid very yellow, contained only 25 white cells, on attempt to puncture the superior longitudinal sinus it was found to be thrombosed, no blood secured but a firm resistance encountered, gradual recovery; well one month later.

D. (A) ANALYSIS OF 100 AUTOPSIES OF HYDROCEPHALICS

Case 1 (Path. 7805, Surg. 51254, 61185)

A. F., born by full term, normal labor, ill on second day, many convulsions, nearly died, right hemiplegia noted several months later, at one year convulsions returned and continued at variable intervals all life.

P. E. At seven years head of normal size and shape; right eye turns

out, spastic weakness of right arm and leg which are smaller than on left, mental development only slightly retarded, can read and write, X-ray of head shows "extreme convolutional atrophy."

Operation: Skull thick, under dura on left side there is a thick pigmented layer of fibrous tissue adherent to dura and to arachnoid; when this is removed pia-arachnoid is thick and opaque, cortex is excessively thin and

TABLE 3

Analysis of pathological anatomy in 100 cases of hydrocephalus

CAUSE	TOTAL NUMBER	BLOCK AT:			
		Iter	Foramina	Base	Undetermined
Meningitis (still active)	34	6*	7	3	18
Adhesions in meninges	19†	0	14	5	0
Syphilitic meningitis	3	0	2	0	1
Occlusion of iter alone	14	14‡	0	0	0
Associated with congenital defect	19§	4	6	1	8
Spina bifida	16	4**	5	1	6
Encephalocele	3	0	1	0	2
Associated with porencephaly	3††	2	0	0	1
Old subdural hemorrhage	2‡‡	2	0	0	0
Fresh ventricular hemorrhage	2§§	2?	0	0	0
Nothing found	4	0	0	0	4
Total	100	30	29	9	32

* Sections of only one, iter "plugged" by inflammatory process.

† Many of these cases were congenital.

‡ Sections of 7 midbrains available: 5 showed "malformed" iters, 1 iter was blocked by transparent septum, these 6 cases were congenital, a "plugged" iter was found in the 7th brain, in this case hydrocephalus followed meningitis.

§ Intracranial hemorrhage was found in 4 of these.

** "Malformed" iter found in the one brain sectioned.

†† Abstracted below.

‡‡ Both show "plugged" iter, abstracted below.

§§ Abstracted below.

scarred; tissue removed for microscopic examination showed fibrous tissue with old yellow pigment, i.e., old organized blood clot, death after second operation to relieve hydrocephaly.

Pathological anatomy. Dura thickened and adherent over left hemisphere, all meninges bound together, cortex of left hemisphere very thin and nerve cells lacking, left ventricle enormous, rt. and 3rd ventricles also enlarged, meninges on right delicate, aqueduct is plugged by a mass of

fibrous tissue, outlines of old aqueduct shown by many small ependymal lined channels, apparently not quite completely occluded, yellow pigment in meninges and ventricles.

Case 2 (Path. 7170, Surg. 57635)

C. S., born by normal labor, severe illness in first few days, left hemiplegia noted when child was a few months old. At six years developed left-sided convulsions, which continued.

P. E. At seventeen years head of normal size and shape, slight left hemiplegia with under development of left arm and leg, vision poor, moderate optic atrophy, nystagmus; X-ray of head showed marked "convolutional atrophy;" mental development nearly normal; very irritable at times.

Operation: Meninges greatly thickened and adherent over right hemisphere, cortex reduced to a shell, dense adhesions at the base. Death.

Pathological anatomy: Bilateral internal hydrocephalus, right temporal lobe reduced to 5 mm. thickness, merely glial tissue, no nerve cells, meninges thickened and adherent to cortex, left hemisphere shows only dilatation of ventricle, cortex averages 2 cm., meninges over left convexity are normal, iter is occluded by fibrous plug which is quite yellow, small channels lined by ependyma around margin of plug.

These two cases are almost identical. The fact that the head was of normal size is against the possibility that these were cases of congenital hydrocephaly with birth injury. The "plugging" of the iter is also against that supposition. However, it is possible that a hemorrhagic encephalitis, or perhaps a local meningitis, in the first few days might have produced this picture.

Case 3 (Ped. 45349, Path. 8785)

M. W., first child, born at term, difficult delivery, in labor thirty-six hours, cried at once, nursed well for week, then stopped, vomited blood, blood in stools, very ill.

P. E. At two weeks, head 37 cm. sutures separated, bulging fontanels irregular respiration, bilateral internal squint, weak and flaccid, ventricular puncture difficult, ventricles small, contain altered blood, cistern and spinal puncture also give altered blood, several transfusions, pressure symptoms vary; at one month head only 38 cm. ventricles large, death.

Pathological anatomy: Moderate internal hydrocephalus, old blood in ventricles and subarachnoid spaces, iter full of clot.

The intracranial hemorrhage was probably due to hemorrhagic disease

rather than to birth injury. However, the fact that the ventricles were small at first and the head enlarged slowly as compared to case 4, makes it probable that the hemorrhage was the cause of the hydrocephalus. It is questionable whether this child would have developed a chronic hydrocephalus if it had survived the acute stage.

Case 4 (Ped. 45267, Path. 8811)

A. R. K., fifth child of healthy parents, born at term by normal labor, head normal size, resuscitation difficult, never nursed well, vomited frequently.

P. E. At seven days head 37 cm.,some twitching of right arm, altered blood in cistern magna; at twelve days head 40.5 cm. vomited frequently, death on twenty-sixth day.

Pathological anatomy: Extreme internal hydrocephaly, adhesions under base, blood in ventricles and subarachnoid spaces.

The rapid enlargement of the head suggests that this was a case of congenital hydrocephalus with a birth injury.

THREE CASES WITH PORENCEPHALIC CAVITIES

Case 5 (Ped. 17182, Path. 5542)

Baby S., born by full term normal delivery, breathed poorly, ill from birth, some blood in the stools, possibly hemorrhagic disease.

P. E. At thirteen days head 39.5 cm., altered blood in the spinal and ventricular fluid, small encephalocele presenting through the anterior fontanel, blood Wassermann negative; at nineteen days head was 39.7 cm. in circumference; death at five weeks.

Pathological anatomy: Moderate thinning of the cortex, small cavity in left frontal lobe separated from ventricle by a perforated membrane, this corresponded to the position of the encephalocele; no explanation found for the hydrocephalus; no sign of syphilis; no spirochetes found.

The rapid enlargement of the head strongly indicates that the hydrocephalus was present at birth, and the intracranial hemorrhage was an accidental complication.

Case 6 (Ped. 13824, Path. 5098)

G. H., only child of normal parents, birth by prolonged labor; two doses of pituitrin given, no forceps; head measurements unknown, stuporous on seventh day, grew stiff, stopped nursing, vomited, cyanotic at times until age of three months when vomiting started again.

P. E. At four months head 44 cm. in circumference; marked general spasticity of extremities, more severe in arms; optic atrophy, no sign of mental development, death at five months.

Pathological anatomy: Gross, symmetrical cavities in both hemispheres; anteriorly these cavities are continuous with the ventricles and the cortex is reduced to a few mm. in thickness; posteriorly the ventricles are not very large and the cavities are separated from the ventricles by a thin membrane—these cavities are lined by a rough glial surface; aqueduct is occluded, no sections could be found but from the description it seems that the aqueduct is "plugged."

Case 7 (Ped. 42899, Path. 8436)

J. W. J., breech delivery, resuscitated with difficulty, always irritable and fretful, at one month was noted to be rigid and head was retracted, head seemed large at this time; at eight months twitching of the face and hands, vomiting and apparently headache.

P. E. At six years, deaf-mute, head 53 cm. in circumference, bilateral choked disc, tremor of head and both arms, roentgenogram showed signs of increased pressure, blood in ventricular and spinal fluids, "phthalein" test indicated obstructive type.

Operation: Aqueduct pin point aperture, dilated, no cause for bleeding found; hyperthermia and death.

Pathological anatomy: Bilateral papilledema, moderate hydrocephalus, multiple extra-ventricular cavities in temporal and parietal lobes,—these are bridged across by many fine trabeculae containing blood vessels, and lined by a rough glial membrane, only a thin layer of tissue separates them from the ventricles; the internal capsules are not interrupted; no signs of meningitis; aqueduct now patent because of surgical dilatation.

(B) Clinical Analysis of 100 Cases of Hydrocephalus

Case of hydrocephalus possibly due to birth injury

Case 1 (Ped. 24513)

E. H., third child of healthy parents, normal labor, no head measurements obtainable, convulsions at five days, development retarded in every way, another series of convulsions at three years.

P. E. At five years head was 55 cm. in circumference, right leg and arm and left leg spastic; severe mental defect, inability to speak; history unreliable, family ignorant and illiterate, diagnosis at least questionable.

CASES OF HYDROCEPHALUS PROBABLY CONGENITAL ASSOCIATED WITH BIRTH INJURIES

Case 2 (Ped. 35967, Obs. 12319)

J. C., second child, first pregnancy resulted in miscarriage, born by difficult version and extraction, hard to resuscitate, fractured skull and cephalhematoma, head measured 34 cm. sub-occipito-bregmatic at birth. P. E. At one month head was 43 cm. in circumference, not quite

TABLE 4

*Clinical analysis of 100 cases of hydrocephalus**

	NUMBER	
Syphilis	3	
Meningitis (observed)	19	
Meningitis (history)	17	
Associated congenital defect	23	
Spina bifida		12
Encephalocele		7
Chondrodystrophia		2
Multiple bone defects		1
Scaphocephaly		1
Brain tumor	3	
Family history of hydrocephalus	1	
Possibly due to birth injury	1†	
No cause found	33‡	
	100	

* No autopsies, several operations.

† Abstracted below.

‡ In 15 of these cases the history stated that the head was enlarged at birth, exact measurements usually not available. In 4 of these 15 there was evidence of birth injury. These are abstracted below.

symmetrical, at two years and three months head was 58 cm. in circumference, some weakness of left leg, tendon reflexes increased on both sides; mental development somewhat retarded; patient was over weight and larger than the average for age, hydrocephalus seemed arrested or only slowly progressive.

Case 3 (Ped. 41412)

C. B., third child of normal parents, born at full term by non-instrumental labor, nursed poorly, respiration feeble, several convulsions on fourth day, enlarged head noted at birth.

P. E. At two months head enlarged not quite symmetrical, partial left hemiplegia, death at nine weeks.

Case 4 (Ped. 22399)

C. R., third child of healthy parents, born by precipitate labor, head noted to be large and bones soft at birth, at three weeks head was greatly enlarged and "round," always nursed poorly, and was unresponsive, at seven weeks hydrocephalus was diagnosed.

P. E. At two months head was 47 cm. in circumference, little or no spasticity, ventricular puncture showed old blood in the ventricles, non-communicating type, at three months head was 49 cm. in circumference, left hemiplegia was noted, convulsions, death at six months no autopsy.

TABLE 5

Analysis of 8 cases of congenital spastic paralysis in hydrocephalics due to birth injury

	NUMBER
Monoplegia	1
Hemiplegia	5
Triplegia	1
Double hemiplegia (porencephaly)	1
	8

Case 5 (Surg. 53834)

G. F., born by difficult, instrumental labor, injury on left temple and over left eye, paralysis of left arm and leg, some squint, general muscular twitching, head 41 cm. at birth, rapidly enlarged since.

P. E. At three years internal squint of left eye, right pupil large and fixed, partial left hemiplegia, bilateral extensor response, head 56 cm.; moderate mental defect.

E. Analysis of 8 Cases of Focal Eiplepsy from the Surgical Records with Operative Notes

Case 1 (Surg. 41240)

R. L. G., born by difficult labor, head injured by forceps; no paralysis noted; general convulsions since the age of seven years, mental development has been fairly good.

P. E. At age of eleven years head shows bulge in right fronto-parietal region, examination otherwise negative, no paralysis.

Operation: Right craniotomy; bone thin, few dural adhesions, large yellow cyst replacing upper half of frontal lobe full of yellow fluid.

Microscopic examination shows wall of cyst is composed of glial tissue covered by arachnoid; old blood pigment in wall.

Case 2 (Surg. 40710)

W. U. D., first child of healthy parents, born by long difficult labor which was terminated by high forceps, head cut by forceps, never used right leg normally; focal convulsions since age of one year.

TABLE 6

Analysis of 8 cases of focal epilepsy probably due to birth injury

	NUMBER
*Birth history:**	
Convulsions in first two weeks	0
Feeble nursing and respiration	2
Difficult labor	6
Precipitate delivery	0
Prematurity	0
Heads:	
Microcephaly	0
Asymmetrical heads	3
Paralysis:	
Monoplegias	2
Hemiplegias	5
Hemianopia	1
No paralysis	1
Pathological anatomy:	
Intracerebral cysts	3
Local cortical atrophy and scarring with thickening of overlying pia-arachnoid	3
Organized subdural clot with atrophy of underlying cortex	1
Nothing found at operation	1

* No history of birth was secured in 2 cases. These histories were obtained in most cases many years after birth and consequently do not obtain as much information about early infancy as the Harriet Lane Home histories.

P. E. At the age of three years head normal size, depressed area in left frontal-parietal region; right leg somewhat spastic, mental condition slightly subnormal.

Operation: Left craniotomy showed very vascular dura, and edema of the arachnoid, no other lesion found.

Microscopic examination of bit of arachnoid removed showed normal meninges.

Case 3 (Surg. 41660)

W. H., born by difficult labor, instrumental extraction, very difficult to resuscitate, head "injured;" always weak on right side, focal convulsions since age of 18.

P. E. At age of twenty-two head normal, mental condition slightly subnormal, severe right hemiplegia, underdevelopment of right arm and leg.

Operation: Left craniotomy, dura adherent to arachnoid, thickening of pia-arachnoid, atrophy of convolutions especially the precentral gyrus, large collections of yellow fluid in Sylvian fissure.

Microscopic examination of piece of arachnoid removed showed great fibrous thickening and no sign of an inflammatory process.

Case 4 (Surg. 50719)

C. D. P., second child of healthy parents, no definite history of birth obtained, never used left arm and leg normally, walked and talked late, infrequent convulsions on the right, always able to do his school work but not brilliant.

P. E. At thirteen years in eighth grade, weakness and under development of left arm and leg, left homonymous hemianopia, slight sensory loss on left side.

Operation: Thin walled cyst in Sylvian fissure and right parietal region full of clear fluid, small opening into ventricle, meninges over this cystic area are greatly thickened and adherent.

Case 5 (Surg. 51428)

S. E. W., born by difficult forceps delivery, no injuries noted at birth, at age of two years focal convulsions started on the left side and a variable left hemiplegia was noted, mentally child progressed well.

P. E. At five years head normal, seemed intelligent, left hemiplegia always severe after convulsion, but almost imperceptible at other times.

Operation: Dura thickened and adherent to a thick layer of fibrous tissue beneath it, this tissue was full of yellowish cysts and also adherent to the arachnoid, pia-arachnoid thickened and cystic, moderate atrophy of convolutions beneath these cysts, central gyri involved most severly.

Microscopic examination of the layer of tissue found under the dura

showed only fibrous tissue and yellowish cysts, no signs of an inflammatory process, or of neoplasm.

Case 6 (Surg. 55411)

J. P. M., second child, birth normal as far as is known, never used right arm and leg well; focal convulsions at four years, more frequent since eight years, mental development fair, did moderately well at school.

P. E. At seventeen years head normal, right arm spastic and under-developed, right leg shows very little weakness; slight mental defect.

Operation: Dura normal, huge cavity traversed by trabeculae, no communication with ventricle, opens into Sylvian fissue.

Case 7 (Surg. 63376)

W. V., first child, forceps delivery, very difficult; head deformed, right eye prominent, left eye sunken in, walked at twenty-two months, talked at two years and six months; focal convulsions on left at two years; left arm and leg small; after each convulsion complete left hemiplegia.

P. E. At eight years head large, large depression to right of sagittal suture; left arm and leg smaller than right, but strength only slightly diminished, some mental defect.

Operation: Dura adherent over right hemisphere and thickened, pia-arachnoid thickened, central convolutions atrophied; in this area cortex was only one cm. thick and scarred and dense.

Case 8 (Surg. 64089)

T. S., born by difficult delivery, high forceps, difficult to revive, deep cyanosis; walked at fourteen months, but never used right arm or leg well; focal convulsions on right at nine years; right hemiplegia after each attack, mental development fair; in seventh grade at twelve.

P. E. At thirteen years head normal; slight spastic weakness of right arm and leg, right arm and leg under-developed.

Operation: Pia-arachnoid thickened over central gyri and angular gyrus, convolutions atrophied and scarred, microscopic examination showed extensive scarring of cortex and thickening of meninges, old blood pigment.

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John Howland

February 3, 1873- June 20, 1926

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Many persons who are very gifted and who devote themselves without stint to their tasks in life gain grateful appreciation of their service to mankind and of their betterment of knowledge. To only a few, however, is given in addition that warmth of enthusiasm and of human sympathy that kindles the divine fire in others and thus, through the inspiration that such natures inevitably give, carries on their influence when they are gone.

Gracious personality, elevated character, distinguished intellect; all these are needed to make the most effective men. There comes occasionally into one's life a personality that excites an instant sense of vivid, eager, gracious charm. Sometimes it proves that these magnetic gifts are worn only as an outer garment and only in the superficial relations of life. With rare individuals there is an equally immediate and magnetic feeling that these qualities come surely from within and are founded upon still more precious things; generosity, sincerity, upright truth.

John Howland's original American forbear and his fellow Pilgrims brought to this land unbending devotion to the right as they saw it. This frequently passed down to their descendants. It has often lent to their view of the proper conduct of life a touch of severity that sometimes tries natures of softer kind but that goes far toward explaining the large part many of them have played in fine progress. A share of this Howland had in severe judgment of himself and his work, in relentless, patient purpose and determination. It had to do with the untimely end; sense of duty made him wholly unremitting in work and unsparing of himself.

But in him the Puritan was dominant only in his sense of responsibility. He had other spiritual ancestry. He owed

> To one the cool and reasoning brain,
> To one the quick, unreasoning heart.

He chose work with little children not merely as an interesting and fruitful field of effort, but because he loved to minister to their dependence and innocence. He sought and made and held friends ardently, loyally and with outgiving spirit.

He loved the beautiful, particularly the out of doors, and especially the lovely New England hills that his ancestors came from and amongst which he now lies.

As a companion or teacher he was vivid, humorous, stimulating, full of imagination, informed in many and wide ways.

He rejoiced in books, especially in books of biography and history of the very human kind; and in medicine and related science he sought with eagerness and appreciation the information that reading gives. As a teacher it was a joy to him to carry this on to others and his own happiness in scholarly things and his generous spirit of service to others were the origin of this journal. To it, taxed as he was by other duties, he devoted constant thought, patient, detailed effort and unending enthusiasm. The idea that led to it was his, as were the heavy efforts necessary to carry it past the inevitable early difficulties of so unusual an undertaking; and it was his broad knowledge that recognized which discussions were needed and which would be fruitful.

With such gifts as he had, with intense industry, with high purpose, with joy in human service it was but natural that he had no superior as a physician to children, that he inspired students and staff, and that he left disciples in high places in many parts of the land. Beyond these accomplishments he added to knowledge in ways that will endure, and he began and determined throughout this country, a transformation in the methods of studying, of teaching and of investigating the subject that was his life's work.

On the entrance to one of our universities is the inscription:

Enter this gateway and seek
The way of honor
The light of truth
The will to work for men.

He entered life treading the way of honor, he sought ever the light of truth, the will to work for men controlled his life.

ACTIONS AND USES OF THE SALICYLATES AND CINCHOPHEN IN MEDICINE

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INTRODUCTION

This summary covers all the available literature since the introduction of the salicylates and cinchophen into medicine, that is, during the period of the last half century. It is based on a previous summary published twelve years ago in the annual (1914) report of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association, and is essentially an amplification with revisions and additions embodying the results of investigations to date. Since the first review, cinchophen has come into extensive use in the treatment of rheumatism and therefore has been included in this review.

The salicylates are among the most extensively investigated drugs, both experimentally and clinically. This is less true of cinchophen. The period from 1875 to about 1880 abounds in contributions dealing with studies on the antiseptic and general pharmacological actions of the salicylates. Their value in rheumatic fever had been recognized as early as 1876, but it is the later and more recent studies that have been concerned more directly with the therapeutic application in rheumatism and other diseases. There is no doubt that the studies of the past decade have changed our views regarding the specificity of the salicylates in rheumatic fever. However, it can not be said that there is as yet a complete agreement on the mechanism of their highly efficient symptomatic relief in this disease. After half a century of continuous and extensive trial, there is, therefore, still considerable need of investigation of their actions.

There are also economic and health aspects to the usage of these drugs which indicate the desirability of all possible information. Probably indiscriminate medication with the salicylates and cinchophen exceedsthe proportions with all other drugs. This is indicated their by extensive popularity in the relief of headaches, colds, neuralgias, etc. Many persons become addicted to the use of small doses over long periods in the treatment of minor complaints, while the dosage in rheumatism and kindred disorders is generally large, or massive. The public health aspect of mass drugging with these agents, therefore, would seem to merit some attention.

The salicylates are the sovereign remedies in the treatment of rheumatic fever, a disease with most serious economic aspects. The Ministry of Health in England (1) has ascertained that nearly one-sixth of the industrial invalidity in that country is due to rheumatism and that the loss to the nation is over three million weeks of individual work from the insured population and an estimated loss of £2,000,000 (about ten million dollars) to the beneficiaries. In this country, the United States Public Health Service (2) has ascertained that acute rheumatism was third in the list of sixteen principal causes of disability in 1924 among male wage earners in different industries. The number of cases was exceeded only by one disease, namely influenza, as the first principal cause, and by non-industrial accidents as the second cause. Rheumatism is also one of the most important causes of cardiac disorders. Falkner and White (3) estimate that 95 per cent of the cases of heart disease in young people can be traced to rheumatic fever and chorea. Despite the therapeutic use of these drugs in rheumatic fever, there remains the large incidence of cardiac disease directly traceable to it. This naturally raises the question of their therapeutic efficiency in this disease, which merely raises the ultimate question of how well their actions and uses are understood, their limitations, the possibilities of doing more harm than good, etc. Therefore, if this review will add to the knowledge and assist future investigators of these drugs, the effort spent upon it shall have been adequately compensated.

In general, meticulous details have been avoided, but the necessary objective data and results have been cited fully so as to leave no doubt of the basis for judgment or opinion. When decision has been im-

possible, the reader will have at hand at least the available sources of information. Owing to their importance, or to some special interest, certain topics have been purposely discussed in greater detail than others. The chronological order of development of the various topics has been maintained as much as possible, but this order has been waived when the topic merited development from another view point. The references used in the text have been numbered consecutively throughout. Those that have not been used are arranged alphabetically, and have been appended to the bibliography for sake of completeness. The originals of many of the latter were not available; others appeared without merit, and those on salicin were left out because the drug is obsolete.

CHEMICAL AND PHYSICAL

The presence of salicyl in plants, discussed by Désmoulière (4), was known long before the salicylates were prepared synthetically. Salicylic acid ($C_6H_4 \cdot OH \cdot COOH$) was first prepared in 1838 by Piria (5) from salicin, which had been discovered in *Salix alba* (willow) in 1827 by Leroux (6). In 1844, Cahours (7) prepared the acid from gaultheria oil, but it was not until 1860 that Kolbe and Lautemann (8) prepared it synthetically from phenol by a process essentially in vogue today.

The more important salicyl esters represent later developments. Methyl salicylate ($C_6H_4 \cdot OH \cdot COOCH_3$) was first obtained in 1843 by Cahours (9) from oil of gaultheria, but now its chief source is the oil of sweet birch. Acetylsalicylic acid or "aspirin" ($C_6H_4 \cdot O \cdot (COCH_3) \cdot COOH$), which is not an ester, was introduced into clinical medicine by Wohlgemuth in 1899 (10), and shortly afterward, in the same year, its pharmacological action was described by Dreser (11). The word "aspirin" was coined from "spirsäure," an old German name for salicylic acid, the prefix "a" indicating "acetyl." Salicyl salicylate, or salicylosalicylic acid ("salysal" or "diplosal") ($C_6H_4 \cdot OH \cdot COO \cdot C_6H_4 \cdot COOH$), was introduced by Boehringer and Soehne in 1908.

The physical and chemical properties of salicylic acid, sodium salicylate and acetylsalicylic acid are so well known that they require no description here. They may be found in the Tenth United States Pharmacopoeia (1926) in which these preparations are official. Salicyl

salicylate and some other minor derivatives are described in New and Nonofficial Remedies (12). It should be borne in mind that the salicyl content of the different preparations differs considerably. The percentage yield of salicylic acid is as follows: sodium salicylate, 85.6 per cent; methyl salicylate, 90.7 per cent; acetylsalicylic acid, 77 per cent, and salicyl salicylate, 106.2 per cent. Among other contributions dealing with the chemistry of the salicylates are the older ones by Gerland in 1852 (13), Proctor in 1844 (14) and Bourquelot in 1894 and 1896 (15) and the more recent ones by Hilpert in 1913 (16) and Savorro in 1914 (17), and of Furukawa in 1919 (18) on solubilities.

The differences in structure and composition of the compounds suggest differences in their pharmacological actions. These will be considered later on. According to Dreser (11), the sweetish taste of salicylic acid depends on the hydroxyl group (the phenolic portion), and its taste and medicinal virtues are lost by replacing the hydrogen of the hydroxyl by the methyl group, but only the taste on substituting the acetyl group. For this reason, acetylsalicylic acid is not sweet. The methyl combination is too firm to cause physiological action.

The solubility and hydrolysis of the different preparations differ and are of importance in connection with prescribing and gastro-intestinal decomposition and absorption. One part sodium salicylate dissolves in 0.9 part, and one part salicylic acid, in 460 parts of water, while acetylsalicylic acid, salicyl salicylate and methyl salicylate are only sparingly soluble. The acid and ester compounds are freely soluble in alcohol and other lipoid solvents. Acetylsalicylic acid and salicyl salicylate also dissolve in alkalies, decomposing into the salts of their acids according to the strength and nature of the alkali. Such strong alkalies as sodium hydroxide and carbonate decompose (hydrolyze) them rapidly and completely, but this is different with sodium citrate and phosphate. These alkaline salts decompose the salicyl compounds much more slowly.

Becker (19) reported that 4 grams of sodium or potassium citrate dissolve 1 gram of acetylsalicylic acid completely without decomposition as indicated by the absence of the iron-salicyl reaction with the mixture and the removal of sodium acetylsalicylate by shaking it with chloroform and ether. Observing the hydrogen ion concentration in an aqueous mixture of acetylsalicylic acid, 7.5 per cent, and

sodium citrate, 30 per cent, with pH 5.4, Leech (20) found that 50 per cent of the acetylsalicylic acid was hydrolyzed at the end of 4 days, 75 per cent at the end of 9 days and was completely hydrolyzed at the end of 17 days and at this time the mixture had a pH of 4.6. Owing to the fact that acetylsalicylic acid is a fairly strong acid, the alkaline reaction of the citrate in Leech's experiments was changed from pH 9.0 to 5.4. In buffer phosphate mixtures at room and body temperatures, Hanzlik and Presho (21) found the hydrolysis with liberation of salicylic acid to be less rapid. In fact, it was much less marked in neutral than in acid and alkaline mixtures. That is, in neutral mixtures (pH 7.0) it was only about 1 per cent, while in acid (pH 4.8 to 5.8) and alkaline (pH 8.0 to 8.4) mixtures it was about equal, amounting to 5 per cent. The hydrolysis increased with the time amounting to 35 and 45 per cent at the end of 18 hours and 55 and 95 per cent at the end of 24 hours in the same acid and alkaline buffers, respectively. It was not appreciably increased on the addition of bile and the ferments of pancreatin. Neutralization of acetylsalicylic acid by lime water (calcium hydroxide) with the formation of calcium acetylsalicylate is claimed by Altwegg (22) to restrain hydrolysis. The sodium and calcium salts of acetylsalicylic acid would have the advantage of being more pleasant to the taste and less irritating to the stomach than the acid itself when large and repeated doses are used in the treatment of rheumatism. Under the same conditions, salicyl salicylate was found by Hanzlik and Presho (23) to be hydrolyzed more slowly than acetylsalicylic acid, no demonstrable salicylic acid having been liberated in acid and neutral buffer mixtures. In buffers possessing the slight alkalinity of blood (pH 7.4), the hydrolysis was only 3 per cent, and this was increased to 8 per cent at the end of 6 and 24 hours in more alkaline buffers of pH 8.0 and 8.4. These results indicate that the greater proportion of the salicyl salicylate (salicylosalicylic acid) and acetylsalicylic acid existed in these mixtures of wide pH range, covering that of the alimentary tract, as their sodium salts unchanged, and therefore, that considerable absorption of the unchanged derivatives might be expected in the alimentary tract. Methyl salicylate was found by Hanzlik and Presho (24) to be unaffected at the end of one hour in acid and alkaline buffers, and slightly hydrolyzed, i.e., 2 per cent at the end of 16, and 3 per cent

at the end of 24 hours, in the most alkaline buffer mixtures used (pH 8.4). This might be expected, since, according to Furukawa (18), the water solubility is only 0.013 per cent.

The discoloration of solutions of sodium salicylate on standing has been known for a long time but is not understood. Except for inelegance, the change is of no practical importance in prescribing. The products formed do not appear to be toxic. There is, however, a loss of strength in such solutions, and this is due in part at least to the growth of fungi and possibly other organisms, for solutions of sodium salicylate or salicylic acid used as colorimetric standards for estimation of salicyl in foods, body fluids, excretions, etc., do not decompose when preserved with chloroform. Mixtures of bicarbonate and salicylate, frequently prescribed in the treatment of rheumatism, become nearly black and eventually deposit a black precipitate. According to Greenish and Beesley (25), this is due to the oxygen of the air acting in the presence of sesquicarbonate and is prevented considerably by the addition of about 0.013 per cent of sodium sulphite (Na_2SO_3) or bisulphite ($NaHSO_3$), or 1 grain to 8 ounces of the salicylate mixture. All of this indicates that the salicyl group is more readily destroyed than is generally assumed, and is of importance in connection with the fate of the compounds in the body. From the fact that lower forms of life thrive in, and even help to destroy, solutions of salicylic acid and sodium salicylate (26) their inefficiency as antiseptics is suggested.

Chemically, cinchophen or "atophan," is phenyl-quinoline-carboxylic acid ($C_6H_5 \cdot C_9H_5N \cdot (COOH)$). It was described by Doebner and Giesecke in 1887 (27) and introduced into medicine by Nicolaier and Dohrn in 1909 (28). It has a bitter taste and causes gastric disturbance owing to its acid character. Its solubility in water is low but this can be increased with the aid of an equal part of sodium bicarbonate or hexamethyleneamine. The bicarbonate forms the soluble sodium salt and the hexamethyleneamine, presumably the ammonium salt. The sodium salt is relatively non-irritating. A mixture of hexamethyleneamine and cinchophen also contains free formaldehyde resulting from the decomposition of the hexamethyleneamine. Therefore, this mixture, which has been advocated, would have no advantage from the standpoint of gastric irritation

over cinchophen itself. Neocinchophen ("novatophan" or "tolysin") is the ethyl ester of methyl phenyl-quinoline-carboxylic acid ($CH_3 \cdot C_9H_4N \cdot C_6H_5COOC_2H_5$). It is not official. It is an odorless and tasteless yellow powder nearly insoluble in water, dilute alkalies and acids, but very soluble in lipoid solvents. The tastelessness is due to its poor solubility, which also suggests poor absorption.

PHARMACOLOGICAL ACTIONS

Antiseptic properties

This is possessed by the salicylates to a limited extent only, being demonstrable with the acids in fairly high, and uncertain or inefficient with the salts in the highest concentrations. Accordingly they are weak protoplasmic poisons. The action is essentially bacteriostatic and not germicidal. In any case, free salicylic acid and the high concentrations of sodium salicylate necessary for antiseptic action are not demonstrable in any of the body fluids, except possibly in urine after full therapeutic dosage. Nothing is known of the antiseptic actions of cinchophen. The evidence presented below deals exclusively with the salicylates.

Fermentation. In 1874, Kolbe (29) found that fermentation of cane-sugar was arrested by a 0.04 per cent solution of salicylic acid; a 0.4 per cent solution preserved fresh milk for 36 hours; 0.1 per cent salicylic acid interfered with the action of emulsin on amygdalin, with the formation of mustard oil, and it preserved minced flesh for a week. Feser (30) confirmed most of Kolbe's observations and, in addition, found that sodium salicylate possessed properties quite similar to those of the acid, although weaker. Müller (31) found salicylic acid in corresponding concentrations to be less active than phenol in preventing putrefaction of urine, but more energetic in preventing decomposition by amygdalin, sugar formation by liver, and peptic digestion. Schaer (32) observed that salicylic acid only mildly retarded the action of unorganized ferments; the activities of ptyalin and diastase were neither prevented nor weakened. Meyer and Kolbe (33) found that 0.25, 0.5 and 0.75 grams of salicylic acid inhibited 1, 15 and 55 grams of yeast, respectively, in 1 liter of sugar solution. The treated yeast remained inactive and the salicylic acid

did not undergo chemical change. Cresotinic acid behaved about like salicylic acid. According to Neubauer (34), the growth of penicillium is also inhibited by salicylic acid.

Béchamp (35) observed that dilute solutions of salicylic acid modified the structure and suspended the functional activities of yeast, but did not kill them. Kolbe (36) observed that the isomers of salicylic acid, i.e., the meta- and para-oxybenzoic acids, its salts and the esters, salicin and saligenin had no antiseptic actions, but the cresotinic acids acted like salicylic acid. This was confirmed nearly 40 years later by Stockman (37) who studied, in addition, some other compounds with the following results: Salicylic acid in 1:5000 concentration delayed, while 1:2000 inhibited yeast fermentation; sodium salicylate had almost no action, the meta- and para-hydroxybenzoic acids required about ten times higher concentrations to produce effects similar to those of salicylic (ortho-hydroxybenzoic) acid and the following were not antifermentative; salicin (1:250); acetylsalicylic acid (1:250), dimethylsalicylic acid (1:1000) and salicyluric acid. According to Fühner (38), 1 cc. of 0.02 per cent salicylic acid inhibits carbon dioxide production in a mixture containing 3 cc. of yeast suspension, 4 cc. of salt solution ($MgSO_4 \cdot 7H_2O$, 0.2 per cent and $KHSO_4$, 0.3 per cent) and 2 cc. of 4 per cent dextrose. Under these conditions salicylic acid would seem to be more efficient as an antiseptic (concentration of about 1:50,000) than under ordinary conditions. The test is claimed to be valuable in examination for food preservatives. Comparing equimolecular solutions of sodium salicylate (1 per cent) and sodium acetylsalicylate (1.25 per cent) on yeast fermentation, Dreser (11) found that the sodium salicylate was about twice as efficient as the acetylsalicylate in diminishing the production of carbon dioxide. However, when the 2 compounds were tested with *B. coli* in milk, their antiseptic efficiency was about the same. Both compounds caused definite injury to the organisms in both sets of experiments. Taking into consideration the pH, Waterman and Kuiper (39) found that salicylic acid in from 0.04 to 0.06 per cent concentration at pH 4.8 to 4.4 was more efficient than benzoic acid at about the same pH in preventing the growth of penicillium glaucum. The anions were not concerned with the checking influence, since much higher concentrations of the salts were ineffective, and the action

was ascribed to the lipoid solubility of the undissociated molecules of the acids. This is probably the only work on salicyl antisepsis in which the influence of hydrogen ion concentration has been considered.

As for the sodium salt of novaspirin, this was found by Dreser (61) not to inhibit yeast fermentation. The same was observed by Corpene (40) with cresotinic acid which is related to salicylic acid.

Seeds and roots. Knop (41) observed that roots of maize plants and various plant seeds lost their reproductive properties in 0.07 per cent solution of salicylic acid. Salicylaldehyde appears to be more efficient, for Gardner (42) found that 10 parts per million in solution cultures reduced the growth of wheat plants 31 per cent during the first six days and the plants were killed in 50 parts per million. Other crops were affected similarly, though higher concentrations were necessary to cause injury to some. In soil cultures, 25 parts per million injured wheat seedlings. Certain bacterial cultures in which the aldehyde decomposed also did not grow.

Putrefaction of flesh. One per cent salicylic acid was found by Salkowski (43) to prevent the putrefaction of a mixture of putrid ascitic fluid and minced meat (incubated at 25° to 30°C.) for eight days; in more highly concentrated solutions the mixtures were preserved for from four to five weeks. As soon as the mixture became alkaline, however, putrefaction ensued. This antiseptic action was exceeded by benzoic acid. Later, Salkowski (44) observed that 0.1 per cent salicylaldehyde prevented putrefaction and 0.25 per cent acted as disinfectant, this compound being more efficient than salicylic acid. Incubation of the pancreas with broth and phenyl salicylate (salol) was found to remain sterile by Lesnik (45). This was attributed to the decomposition of the ester into phenol and salicylic acid, since incubation of saliva, serum albumin and enzymes with salol resulted in the liberation of salicylic acid. Feser (30) found that flesh rubbed with salicylic acid did not putrefy for several weeks, and a putrid extract of meat when treated with double its volume of 0.4 per cent of salicylic acid solution produced no effect subcutaneously. Feser thought that salicylic acid acts less efficiently on chemical fermentation processes. In this connection, it may be stated that the presence of a salol-splitting ferment in human milk has been reported by Usener (46) on the ground that after administration of the ester,

salicylic acid could be detected in the stomach of a breast-fed infant one hour later.

Bacteria. Using a putrid tobacco infusion as bacterial emulsion, Bucholtz (47) found that bacterial growth could be prevented by salicylic acid (0.15 per cent solution), sodium salicylate (0.4 per cent), ammonium salicylate (0.416 per cent), and methyl salicylate (0.1 per cent by volume). Bacteria were killed in the shortest time by 1 per cent methyl salicylate, after a longer duration by 0.5 per cent solution and also by salicylic acid (0.35 per cent). Cech (48) found that higher dilutions than 0.1 per cent of salicylic acid did not inhibit the growth of bacteria. Koch (49) observed that 1:1500 of salicylic acid completely inhibited the *B. anthrax;* but after a month's application to the spores no harmful effects were observed. Heinz (50) quotes Lübbert to the effect that staphylococci required 1:655 of salicylic acid for complete inhibition. According to H. C. Wood, Jr. (51), the neutralization of salicylic acid by sodium and strontium lessens its bactericidal strength very materially. In his studies of different salicyl compounds, Stockman (37) found that *B. coli* kept in contact with a 1:1000 dilution of salicylic acid for three-fourths of an hour stopped the growth permanently, while the meta- and para-hydroxybenzoic acids required about ten times higher concentrations to produce similar effects. Sodium salicylate had almost no germicidal action and salicyluric acid was entirely inert.

Urine. The putrefaction of urine is delayed after the direct addition or internal administration of sodium salicylate. Binz (52) noted that when carbon dioxide was passed through urine containing sodium salicylate, decomposition took place later than when the urine contained either the salicylate or carbon dioxide alone. The antiseptic effects were presumably due to liberated salicylic acid. Meyer and Kolbe (33) found that the addition of 1:2000 of salicylic acid to fresh urine delayed putrefaction so that the urine remained clear for three days and did not become ammoniacal. Fürbringer (53) stated that daily doses of 1 to 2 grams of salicylic acid rendered a urine acid which had exhibited ammoniacal decomposition in the bladder; and a beneficial effect was produced in the inflamed urinary passages.

Its limited antiseptic properties in urine are due to the fact that the salicylic acid is "bound" by the alkaline phosphate present. Meyer

and Kolbe found that 1 molecule of the disodium phosphate (Na_2HPO_4) "bound" two-thirds of a molecule of salicylic acid. They suggested that its limited antiseptic action in body fluids, in general, was because of their alkalinity. Prideaux (54) found that 0.5:1000 of salicylic acid added to urine would prevent the development of bacteria; 1.5:1000 would preserve it for several months and three to four parts per thousand killed bacteria in an infected urine. Sollmann (55) observed that, after a dose of 1 gram of the sodium salicylate, turbidity was slight or absent in the salicylate urine as compared with marked putrefaction in the same urine before the salicylate was taken. Smaller doses of salol (0.3 to 0.5 gram) delayed putrefaction for about 7 hours. Jordan (56) found that the antiseptic power of salicyl urines of high acidity (by titration) was somewhat greater than could be accounted for by an increase in the acidity alone. Jordan took 20 grains of salicylic acid per day and studied the acidity and putrefaction as well as bactericidal properties of samples of twenty-four specimens of urine. His data indicated that salicylate urine possessing about the same acidity as normal urine required practically once as long to become alkaline and nearly twice as long to become ammoniacal. When incubated with *M. staphylococcus* the urine became alkaline in about 2 to 10 days, depending on the acidity. Jordan stated that the normal acidity of urine is sufficient to liberate salicylic acid from sodium salicylate, but that administration of sodium salicylate only slightly increases the acidity of urine. He suggested that the antiseptic power of salicyl urines may be due to a change of proportions between the salicylic and salicyluric acids.

H. C. Wood, Jr. (57) found the efficiency of salicylic acid and salol as intestinal antiseptics limited, being easily surpassed by betanaphthol, formaldehyde and creosote.

Local action and irritation

The application of salicylic acid causes slow and practically painless destruction of the epithelium of the skin though it causes considerable superficial corrosion of mucosae. It is therefore rather irritating, causing nausea and vomiting when administered internally. For instance, Hodara (58) found that prolonged contact with the skin causes swelling of the epidermis followed by desquamation. The salts

are non-irritating even in high concentrations. The destructive action of salicylic acid on cells is made use of in preparations (ointments, collodions, etc.) for softening corns and warts, and in the treatment of certain skin diseases, alopecia, pruritus, etc. For eczema and dermatitis a 10 per cent ointment has been used by Berkenbusch (59). Repeated application of various salicyl preparations to skin was found by Sauerland (60) to cause hypersensitiveness resulting in eruption and the appearance of dermatitis in untreated areas. However, this is not peculiar to salicylic acid, for similar phenomena have been observed with a variety of irritants including chemical warfare compounds and the phenylenediamines. There is no sensitization in the anaphylactic sense, but rather an increased susceptibility of the skin and its capillaries to stimuli.

The irritant properties of different salicyl acids may be demonstrated by the method of Dreser (61). This consists of immersing tails of live fish into saucers containing the different solutions. A fish tail in tonus remains spread like a fan, but when depressed, it collapses and the fan shape is lost. Using this method, Dreser found that depression of fish tails was caused after immersion into a mixture of 4 cc. of 2.5 per cent sodium salicylate and 3 cc. of 0.25 per cent hydrochloric acid, but the control of hydrochloric acid alone produced no effect, indicating that the depression was due to the salicylic acid. Saturated solutions of acetylsalicylic acid also caused depression but not those of novaspirin. The solubility of the latter was too low for adequate concentration, but there was no doubt that the salicylic and acetylsalicylic acids were irritating.

Absorption

Cutaneous. Salicylic acid and its esters in fatty and alcoholic solutions are readily absorbed from the intact skin. In 1876, Drasche (62) demonstrated that an alcoholic solution of salicylic acid applied to the skin caused the appearance of salicyl in the urine almost immediately. After the application of 20 to 30 grains of salicylic acid in olive oil to the axilla, Randolph and Dixon (63) found that the urines of six patients gave positive iron tests for salicyl. In one case enough salicyl was absorbed to relieve rheumatism. Bourget (64) made a quantitative study on the effect of different fatty bases, and the

effect of such factors as age, sex and disease condition on the cutaneous absorption of salicylic acid as judged by the excretion in urine. It was found that salicylic acid was rapidly absorbed from the skin. The rapidity of absorption and the amount depended on the vehicle, being most effective with lard or lanolin, least with vaseline and glycerol. The skins of old individuals were found to be less permeable than those of the young; also, skins of red haired and blond individuals absorbed more readily than those of dark haired. The skins of females were more readily permeable than those of males. These differences were attributed to differences in skins, but no cognizance was taken of interferences with renal function, as most of the patients were ill with acute rheumatic fever and other febrile conditions. Curative effects were obtained in rheumatic fever only. Linnoisier (65) suggested the volatility of salicylic acid at body temperature as the explanation for its absorption by the skin. According to Schumacher (66), the intact skin of the horse, calf, dog and rabbit is permeable to salicylic acid when applied as an ointment or in alcoholic solution; to sodium salicylate somewhat more readily in the form of ointment. However, Levin's study (67) indicates that no salicyl appears in the blood after the application of salicylate ointments to the skin.

The esters of salicylic acid are absorbed more readily than the acid itself or its salts. Linnoisier and Lannois (68) could detect salicyl in the urine 30 minutes after application of the esters to the skin. According to Floret (69), methyl-oxymethyl salicylate (mesotan) is readily absorbed by the skin, and saponified in the body. Impens (70) found that from 8 to 9 per cent of the methyl salicylate is absorbed; monoglycyl salicylate (spirosal) more readily, and to the greatest extent, that is, about one-sixth to one-fifth of the amount used; amyl salicylate, least effectively. Sauerland (60) found that about 0.5 per cent of the salicyl of the methyl salicylate in vaseline when applied to human skin is found in the urine; about 15.4 per cent with spirosal in lanolin; and 2.5 per cent with saligenin in lard and vaseline. Spirosal is practically non-irritant and can be used undiluted, while mesotan is irritant and should be diluted with one to four parts of olive or cotton-seed oil before application. Joachimoglu (71) reports that 24 hours after inunction of the thigh and leg with 10 grams of "rheumasan" (a soap mixture containing 10 per cent salicylic acid) salicyl was detectable in 10 cc. of urine.

Oral and nasal. Recently, Planelles (72) demonstrated qualitatively the presence of salicyl in urine from the smoke of cigarettes impregnated with salicylic acid and acetylsalicylic acid. The smoke was held in the mouth and nose, swallowing and inhalation being avoided as much as possible. Smoking in vitro of such cigarettes also showed the presence of the drugs in the smoke.

Esophageal. Kuzaya (73) found that introduction of sodium salicylate into the esophagus ligated at the cardia resulted in the appearance of salicyl in urine at the end of 20 minutes, but when injected elsewhere salicyl was not excreted into the esophagus, apparently a case of one-sided permeability.

Gastric. The absorption from the lower portions of the alimentary canal takes place much more rapidly than from the skin, mouth and esophagus. The data on gastric absorption of the salicylates are extremely limited; but Burow (74) asserts that the soluble salts are slowly absorbed, and the insoluble pass unchanged into the intestine. According to Breguet (75) alcohol facilitates the absorption of sodium salicylate from ligated stomachs of guinea pigs as compared with aqueous solutions. Of the esters, salol, being relatively insoluble, is not decomposed and absorbed in the stomach, but becomes immediately decomposed and absorbed when it reaches the intestine (Ewald (76)). Ethyl salicylate is claimed by Houghton (77) to be readily and quickly absorbed from the stomach.

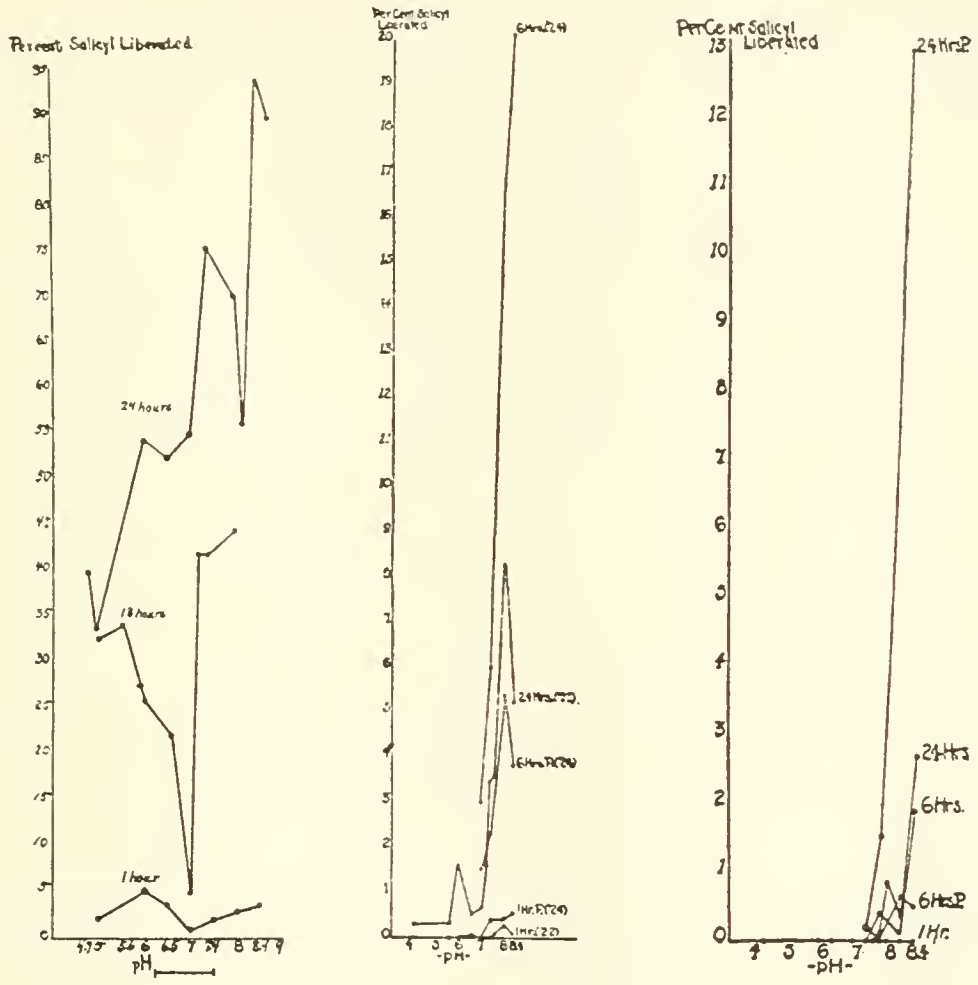
Intestinal. With small doses of the sodium salicylate the intestinal absorption is so rapid that no local antiseptic action occurs, according to Kumagawa (78). The effect of different vehicles on the absorption of sodium salicylate as judged by the excretion in urine was studied by Mastbaum (79). Alcohol did not influence and mucilaginous vehicles delayed the absorption as compared with aqueous solutions. Pinczower (80) finds that the absorption (as judged by the excretion in urine) of salicylic acid, sodium salicylate, acetylsalicylic acid, novaspirin, salol and salipyrin from the stomach and intestines presents no differences worthy of mention.

Nencki (81), Lesnik (45) and Bondzynski (82) thought that the esters of salicylic acid are practically entirely hydrolyzed in the intestine before absorption takes place. However, H. C. Wood and Hare (83) suggested that the greater toxicity exhibited by some of

these esters might be due to the absorption of the esters themselves. Baas (84) claimed that the ethyl and methyl salicylates can pass into the urine combined with the ethereal sulphates, but not free. Convincing evidence that salicyl esters as such pass into the circulation was obtained in their studies on urinary excretion by Hanzlik and Presho (24), who found up to about 0.12 per cent in urine of the clinical "toxic" dose (6.5 to 10.7 grams) of methyl salicylate but probably more was absorbed from the intestine. The urines possessed the odor of wintergreen. These investigators also found that about one-half the total salicyl salicylate and acetylsalicylic acid administered is absorbed, for from 8.8 to 36.6 per cent of the former (23) and 6 per cent of the latter (21) appeared unchanged in the urine. According to Buss (85), no salicyl appears in the urine in one hour after the administration of salicin.

Hydrolysis of esters in the stomach and intestine. The reason for the considerable absorption of unchanged esters is that hydrolysis with the liberation of salicylic acid proceeds slowly in the alimentary tract. This is indicated by the results of the incubation experiments of Hanzlik and Presho (21, 23, 24) detailed under "chemical properties," and also by the excretory results just mentioned. The significant results of the incubation experiments were as follows: Liberation of salicylic acid from acetylsalicylic acid in buffer mixtures of the same degree of acidity as in gastric juice and of alkalinity as in intestinal juices was about equal at the end of one hour, i.e., about 5 per cent, and this was increased to 35 and 45 per cent in the acid and alkaline mixtures, respectively, at the end of 18 hours. About 98 per cent of the drug remained undecomposed at neutrality (pH 7.0) at the end of 1 and 18 hours, and about 45 per cent at the end of 24 hours. Accordingly, liberation of free salicylic acid in the stomach would be expected, contrary to the claims. On the other hand, the claims made for poor gastric liberation of salicylic acid from salicyl salicylate appear to be justified, since the liberation was almost negligible in acid buffers. At the end of one hour, 97 per cent of this ester was left unhydrolyzed in buffer mixtures possessing the alkalinity of blood (pH 7.4), and about 92 per cent at the end of 6 and 24 hours in mixtures with alkalinity (pH 8.0 to 8.4) corresponding to that of intestinal juices. Bile and pancreatin did not increase the hydrolysis. The weakest hydrolysis

occurred with methyl salicylate, for no liberation of salicyl occurred at the end of 1 hour in both acid and alkaline mixtures, and at the end of 24 hours about 97 per cent of the ester remained unhydrolyzed in the most alkaline buffers (pH 8.4) used. Pancreatin increased



Acetylsalicylic acid | Salicyl salicylate | Methyl salicylate

FIG. 1. HYDROLYSIS OF SALICYL DERVATIVES IN BUFFER MIXTURES AT 38°C.

P refers to pancreatin. The bracket (——) indicates the region of weakest hydrolysis of acetylsalicylic acid.

the hydrolysis at the end of 24 hours, but bile restrained it. Thus, there seems to be no doubt that salicyl salicylate and methyl salicylate undergo very limited, and acetylsalicylic somewhat more marked, hydrolysis under conditions of the alimentary tract. The presence of

food would tend to restrain the hydrolytic process, if anything, and therefore, considerable absorption of the unchanged compounds might be expected as indeed is adequately indicated by the excretory results to be described and by certain clinical claims. The curves in figure 1 illustrate the results with the salicyl derivatives tested.

Both cinchophen and neocinchophen are absorbed from the intestine as indicated by the symptomatic relief in various conditions, but nothing is known of the quantitative absorption of cinchophen, and there is some uncertainty about neocinchophen. Barbour and Lozinsky (86) studied the absorption of neocinchophen in three dogs and a patient after oral administration of the drug, which was recovered from the feces. The method was accurate to about 12 per cent with small quantities of the drug. Doses of 0.3 and 0.5 gram per kilo in dogs and the man were completely absorbed, none being recoverable from the feces. Out of 9 grams per kilo administered, only 2.7 grams per kilo were absorbed and with such large doses clumps of the drug were observed in the feces. From their results Barbour and Lozinsky concluded that the maximum limit of absorption was practically identical with the full therapeutic dose, apparently "a new and fortunate peculiarity in pharmacological behavior." The results agreed with the conclusion drawn from pharmacological evidence as to toxicity. However, the peculiar behavior of this drug is probably due to its poor solubility rather than to some providential contrivance.

Rectal, vesical and vaginal. Fiedler (87) found that rectal absorption of salicylate is not as good as gastric, but Massol and Minet (88) report that from 24 to 58 per cent of sodium salicylate is absorbed from the rectum. According to Heyn (89) rectal absorption is good, for salicyl appears in the urine in 15 minutes, and the iron reaction persists for from 48 to 72 hours. Absorption from the rectum is completed at the end of from 24 to 36 hours and the greatest absorption takes place during the first twelve hours as judged by ether extracts of the feces. Lenko and Kryzanowski (90) found it took 25 minutes for salicyl to appear in ureteral urine when sodium salicylate was placed into the bladder. In 1876, Fehling (91) reported absorption of salicylic acid from the vagina. Daily douches with 1:1000 to 1:600 solutions were constantly accompanied by the presence of salicyl in the urine.

Levin (67), judging from the quantity of salicyl in the blood, found

that when sodium salicylate is given intramuscularly, it is absorbed much more readily than when given by the mouth.

Permeability of colloidal membranes. According to Oswald (92), salicylic acid increases the permeability of colloids for electrolytes. This has been confirmed by Hanzlik (93) for loops of living, excised intestine. It was found that a solution of 1 per cent sodium iodide in aqueous solutions of salicylic acid (0.3 per cent), sodium salicylate (2 per cent), phenol (1 per cent), benzoic acid (saturated solution) and magnesium sulphate (5 per cent) increased the diffusion of the iodide through the wall of the excised intestine as compared with the iodide alone. Thus it is seen that the increase in permeability is not specific for the salicylate, for it occurred with an electrolyte and other substances, although the increased diffusion was most marked with the salicylate. The phenomenon is suggestive as a possible basis of the absorption of effusions from joints in rheumatic fever under salicylate treatment.

Distribution

Normal fluids and tissues. After its administration, salicyl has been found in nearly every secretion, fluid and organ of the body. Feser (30) found salicyl in all body fluids except milk; the feces, as a rule, being free from salicyl even after large doses. Drasche (62) was not able to detect salicyl in secretions of the mouth and bronchi. Its presence in human saliva has been reported by de Mussy (94) and Bénoit (95), denied by Bälz (96), Blanchier (97) and Stockman (98). Bercke (99) observed that after administration of 1.5 to 2 grams of salicylic acid to the mother the child's urine gave a positive salicyl test in 2 hours; salicyl was also present in the mother's milk, but absent in the amniotic fluid (blood-free) from 4 patients. Zweifel (100) states that when salicylate was given to the mother from $\frac{3}{4}$ hour to 4 hours antepartum, the placenta and blood of the cord contained the drug, but not the urine of the fetus. According to Pauli (101) salicyl appeared in mother's milk in 24 to 36 hours after the administration of about 2 grams per day; the urine of the infant also gave a positive salicyl reaction. After intravenous injection of the sodium salicylate in dogs, J. Bernard and Ch. Livon (102) and Blanchier (97) detected it in the urine, saliva, bile, pancreatic juice, pericardial, synovial,

cerebrospinal and peritoneal fluids, intestinal juice and urine. Blanchier found the time of appearance to be about 4 minutes in the saliva, 10 minutes in the urine and 30 minutes in bile. Bälz (96) could detect no salicyl in sweat. However, Blanchier quotes Buss, Riess and Geissler as having detected it in sweat, and recently Tachau (103) reports its presence in the sweat of five individuals after 6 gram doses of sodium salicylate; and both phenol and salicyl after 3 grams of salol. Stockman (98) found only traces in sweat, but considerable amounts in synovial fluid. Nencki (104) reported the presence of salicyl in bile, but not in the gastric juice. In cerebrospinal fluid small quantities were detected by Olmer and Tiau (105), and the quantity present appeared to be independent of the quantity administered. After doses of from 0.5 to 1.5 grams per kilo, Suck (106) demonstrated the presence of salicyl in practically all the organs (blood-free) of the rabbit, cat and dog. Its presence in cerebrospinal fluid was denied by Rotky (107). Fillippi (108) detected salicyl in synovia, brain and spinal cord. According to Vinci (109) the kidney, liver and gastric mucosa contained more of the salicyl than spleen and muscles; and it was present in the brain only after large doses.

I was able to confirm this difference between the brain and other organs in some unpublished experiments on the distribution of sodium salicylate in dogs and cats injected intravenously with doses ranging from 0.18 to 0.4 gram per kilo. It was invariably true that the brain yielded from one-seventh to one-half of the percentage concentration in the blood and the spleen, and about one-sixth to one-third of that in muscle and intestine. Organs rendered blood-free by perfusion gave the same results. The organs were extracted until salicyl-free, and several residues gave negative tests for salicyl. So far as the tests went, the results were clear, but it is possible that the brain only yielded less salicyl because it retained more than the other organs. In such extractions there are many difficulties with the lipoidal and extractive materials in recovering the salicyl, though control organs to which the drug was added directly gave quantitative yields with different compounds by the same method. After gastric and intestinal administration of methyl salicylate and acetylsalicylic acid, essentially the same differences were found between the brain and other organs. This is somewhat surprising in view of the greater lipoid solubility of these two compounds than of sodium salicylate.

Whitham (110) reported the presence of salicyl in fluids of the eye (lachrymal and tears), and it seemed to be increased after hexamethyleneamine, paracentesis, and the application of 10 per cent dionine.

Pathological fluids. Salicyl has been found in both exudates and transudates and in inflamed tissues. Drasche (62) stated that he could not detect it in ascites and in echinococcus fluids. However, later investigators (Rosenbach and Pohl (111); Leuch (112)) repeatedly detected it in hydrothorax, pleural and peritoneal fluids, ascites, and edema fluid of extremities. Its presence in blisters produced by cantharides was shown by Devoto (113) and by Aulmont (114), who also detected it in bronchial secretion and pleural effusion. The presence of salicyl in pleural effusion was reported by G. Dock (115), and in bronchial secretion by Armstrong and Goodman (116). In a study of the distribution in experimentally infected eyes of rabbits and cats, S. R. Gifford (117) found the drug in both the normal (control) and infected organs and the concentration in each was the same. The sodium salicylate was administered gastrically. The concentrations in the whole eye ranged from 1:5900 to 1:4000 and in the aqueous humor, from 1:3600 to 1:250. The time of appearance in the aqueous humor was from 25 to 45 minutes, and enough for estimation was present at the end of 40 minutes. After a dose of 1 gram, the salicyl was present in the aqueous humor after 20 hours, but not after 24. The distribution in the different parts of the eye was as follows: most salicyl was found in the aqueous and vitreous humors, none in the lens and only small amounts in the uveal tract and corneoscleral coat. The drug was found in the aqueous humor of 1 out of 2 patients receiving ordinary doses. In 4 rabbits and 1 cat a higher concentration of salicyl was found in the aqueous humor than in blood, but it may be doubted if the method of estimation in such small quantities of material is accurate enough to make the difference significant.

Arthritis. According to Fillippi and Nesti (118), in 1 hour after acetylsalicylic acid was given by mouth to a rabbit the joint fluid gave a positive salicyl reaction; 2 hours after 4 doses of 0.5 gram each, the joint fluid of a patient gave a stronger reaction than the urine. The same authors observed only a trace of salicyl in fluid of ascites and none in saliva. Bondi and Jacoby (119) reported that the joints of rabbits infected with staphylococci contained more salicyl than those

of healthy animals. Their evidence cannot be regarded as conclusive, since the blood and serum contained the highest proportion of the salicyl, and this would necessarily have to be considered in connection with infected joints with serous effusions. Moreover, the method used for estimating the salicyl was not quantitative. The suggestion that diseased joints may have a predilection for salicyl has recently been tested by Fröhlich and Singer (120) under different experimental conditions. These investigators produced arthritis by local application of mustard and croton oils to the joints of rabbits, using the opposite untreated joints as controls. After administration of large doses of sodium salicylate, the salicyl content of all joints freed from soft tissues was determined by the distillation-colorimetric method. Under these conditions, there was no difference in salicyl content of the inflamed and normal joints; in fact, the inflamed joints of some rabbits contained no demonstrable salicyl. Accordingly, Fröhlich and Singer concluded against any favorable influence of diseased joints on the distribution of the drug. Although in their reply Bondi and Jacoby (121) do not accept the work of Fröhlich and Singer, claiming quite justifiably that the conditions of the two sets of experiments are not comparable, there is no doubt at least that Fröhlich and Singer worked with diseased (inflamed) joints and that much cannot be said of the systemically infected animals of Bondi and Jacoby. Bondi and Jacoby's criticism of the time of bleeding in Fröhlich and Singer's experiments does not seem valid, since the control and inflamed joints were obtained and analyzed at the same time. A safer way of testing the selective distribution of salicyl in joints would be to compare the salicyl content of the blood and joint fluid of the same individual. This was done by Scott, Thoburn and Hanzlik (122) on patients suffering with rheumatic fever and receiving full therapeutic doses of sodium salicylate. The salicyl content of both fluids did not differ markedly, being somewhat less in the joint fluid as might be expected. A concentration of 0.02 per cent was found in blood and 0.018 per cent, in joint fluid. Hence, for all practical purposes, the alleged selectivity of salicyl for inflamed joints does not exist. If anything, the results of Fröhlich and Singer and of Scott, Thoburn and Hanzlik suggest that the swollen and inflamed membranes of the synovia act as barriers to the diffusion of the salicylate.

Concentration in the blood of rheumatic and normal individuals. This is somewhat less in the blood of rheumatic than of normal individuals. Using full therapeutic doses of sodium salicylate by mouth equivalent to about 13 grams expressed as salicylic acid, Scott, Thoburn and Hanzlik (122) found that the concentration in normal individuals ranged from 0.018 to 0.035 per cent, average 0.0265 per cent, while in rheumatic fever patients it ranged from 0.014 to 0.031 per cent, average 0.021 per cent. The theoretical concentration estimated was 0.02 per cent which compared favorably with the concentration found in rheumatic patients. Probably the lower concentration found in rheumatic individuals was due to some destruction of the salicyl to be discussed under "excretion." After the administration of 1 gram sodium salicylate to a normal person, Fiessinger and Debray (123) report the following percentage concentrations in serum; 0.004 to 0.005 at the end of 10 minutes, 0.05 to 0.006 at the end of 30 minutes, 0.01 at the end of one and one-half hours, and 0.001 per cent at the end of 18 hours. Administration of the drug in capsules did not change the results.

Binding power of the blood for salicylic acid. This has been studied recently by means of extraction and dialysis experiments, and the results seem to indicate some binding of salicyl by blood proteins, varying somewhat with the source and condition of the blood, as in allergy. Van Leeuwen and Drzimal (124) found that the addition of 0.01 gram of salicylic acid to 10 cc. of defibrinated beef blood yielded no ether extractable salicyl, but with 0.015 gram, 0.03 mgm. was extracted and with 0.02 gram, 1 mgm. Normal human blood gave the same results except that the extraction limit was lower (average) with 0.01 gram added to 10 cc. blood, while the blood of asthmatics tested for hypersensitivity to acetylsalicylic acid gave regularly a still lower extraction limit, that is, about 4 mgm. to 10 cc. of blood. From dialysis experiments, it was shown that salicylic acid in serum dialyzed less completely than from water or Tyrode's solution. The quantity held back by normal and asthmatic serums was from 0.072 to 0.9 mgm. in 5 cc. serum while serums of asthmatics hypersensitive to acetylsalicylic acid held back much less, the figures, however, not being given. Seventy-eight hundredths of a milligram was bound by 5 cc. of beef serum and 1.08 mgm. by serum of normal persons and asthmatics,

and the addition of hydrochloric acid did not liberate more. It is claimed by the authors that the results indicate that in asthmatics the binding power of blood for salicylic acid is reduced and there is a higher concentration of the free drug exerting a more pronounced action than if it were bound. Moreover, they think the phenomenon is of significance for the allergic state in general. If their results were more striking and convincing, the argument might have some basis.

The peculiar binding of salicyl by blood plasma has been shown by Chabanier, Lebert and Lobo-Onell (125) in another way. Using collodion sacs, they observed a rapid and almost complete disappearance of salicyl from 0.9 per cent sodium chloride dialyzed against plasma, while only a very small proportion of the salicyl dialyzed from salicylated plasma into untreated plasma. Furthermore, when 10 cc. of serum containing 0.01 per cent sodium salicylate was dialyzed against 0.9 per cent sodium chloride containing the same concentration (0.01 per cent) of salicylate, the sodium chloride-salicylate mixture was found to contain only a trace of salicyl at the end of 180 to 300 minutes. The dialysis into serum was greater the greater the volume of serum. In other words, the serum attracted salicyl despite the original equal content. No attention was paid to the pH in these experiments, a factor that could conceivably affect the result. In later experiments, the same authors (126) claim to have demonstrated further that salicyl is adsorbed by the blood proteins and that its excretion is independent of any renal threshold. On the other hand, Coquonin (127) found that dialyzing salicylated serum against physiological salt solution free from salicyl, or containing the same concentration as the serum, gave an even distribution of the salicyl after sufficient time. Friedrichsen (128) demonstrated a difference in the distribution of salicyl between the corpuscles and serum in beef and rabbit blood. Working with quantities of 0.2 to 0.6 gram salicylate he found a higher concentration, i.e., 1.2 per cent, in serum than in the corpuscles, i.e., 1.0 per cent, a difference that might explain the results in the bloods of van Leeuwen and Drzimal, for probably the condition of the corpuscles in vitro has some influence on their binding power with salicylic acid, which in turn would influence the results of extraction and dialysis. In any case the results

of these dialysis and extraction experiments do not settle the question of the form in which salicyl exists in the circulating blood after administration.

Form in which salicylates exist in body fluids. From the modern conceptions of the reaction of body fluids, it would appear quite probable that the salicylates circulate as the sodium salts. This was thought to be the case by Salkowski (43) and by Meyer and Kolbe (33) as early as 1875. Meyer and Kolbe thought that salicylic acid, when taken internally, was neutralized by the alkaline phosphate and other alkalies of the tissues. Two hundred cubic centimeters of fresh dog's blood was found by them to "bind" chemically about $\frac{2}{3}$ gram of salicylic acid. Taken by mouth, the salicylic acid is first neutralized by the alkaline juices of the intestine and, therefore, is absorbed chiefly as the sodium salicylate. However, it was claimed by Binz (52) in 1876 that the high tension of carbon dioxide of the blood and other fluids in rheumatic fever liberated free salicylic acid which, he believed, was responsible for the therapeutic benefits. The evidences from experiments in vitro offered by him were as follows: Carbon dioxide shaken with a 1 per cent solution of sodium salicylate gave up to ether a substance which gave a positive salicyl test with iron. Urine containing sodium salicylate and free carbon dioxide did not putrefy as readily as when it contained either salicylate or carbon dioxide alone. Salicylic acid crystals were present in two specimens of highly acid urine. It was argued by him that lactic and other fatty acids assisted in the liberation of salicylic acid. From later experiments, Binz (129) concluded that sodium salicylate acts as an energetic bactericide and is destructive when an alkaline bacterial medium is impregnated with as much carbon dioxide as corresponds to that of inflamed tissues in man. The experiments were performed as follows: Under a pressure of half an atmosphere of carbon dioxide about 20 per cent of the gas was dissolved in an alkaline mixture, consisting of sugar, potassium phosphate, ammonium tartrate, and 0.5 per cent of sodium salicylate, and infected with bacteria. This remained sterile for four months at summer and room temperatures. The same experiment performed without salicylate showed an extensive bacterial growth in one week. When the experiment was performed with salicylate, but without carbon dioxide, putrefaction and bacterial

growth occurred in a few days. When the mixture (containing salicylate) was impregnated with about 20 per cent of air, or if the whole was exposed to air without pressure, turbidity and putrefaction resulted in both cases.

Köhler (130) observed that when salicylic acid was dissolved in normal blood, no salicyl was given up to ether, but when blood of asphyxia was used the ether extract contained salicyl. From experiments on animals, Köhler concluded that salicylic acid existed in the blood as sodium salicylate, but that in death from salicylate poisoning the carbon dioxide content of the asphyxial blood was sufficiently high to liberate free salicylic acid, because such blood gave up salicyl to ether without the further addition of acid.

The carbon dioxide (and other acids) theory was strongly opposed by Feser and Friedberger (131) on the ground that drawn blood containing salicylate would not yield any of it to ether until further acidified. These observers advanced the theory that salicylic acid exists as an albuminate in the blood. However, according to Fleischer (132), who also held the opinion that salicylic acid as such does not exist in the blood, even combination with proteins is improbable, since the digestion of blood with salicylic acid resulted in a precipitate and the filtrate contained practically all of the salicylic acid. By digesting salicylic acid with proteins, i.e., albumin, casein and syntonin, or fibrin, and after shaking out the digested mixture with ether, and treatment with boiling water, Farsky (133) found that a residue remained which contained 14.2 per cent of salicylic acid, and the formula $C_{72}H_{112}N_{18}SO_{22}$ was assigned to it. It was found to be soluble in gastric juice with liberation of salicylic acid. Marmé (134) produced fever in a dog and administered sodium salicylate. The venous blood was then extracted with ether, but no trace of free salicylic acid was found.

Still another view was advanced by Jacoby (135). Jacoby believed that salicylate in the blood serum of injected animals existed in a different form than when salicylate was added directly to serum outside the body. The view was based on the following evidence: The blood serum of rabbits previously injected with sodium salicylate yielded salicylate containing precipitates with ammonium sulphate, but the addition of salicylate to serum gave precipitates free from

salicylate. In short, salicylate in the circulating blood seemed to be attached to the proteins. Jacoby suggested that the salicylate may be bound in some way with amino compounds, or proteins resembling them, in the intestine.

The question of the form in which salicyl exists in the circulating fluids and the tissues of the body touches on an important point, namely, the mechanism of the action of salicylates in acute articular rheumatism. The etiology of rheumatic fever is not absolutely settled, but assuming that the condition is a bacterial infection, and since salicylic acid is more bactericidal than sodium salicylate, and from the fact that carbon dioxide can liberate the acid from sodium salicyl-

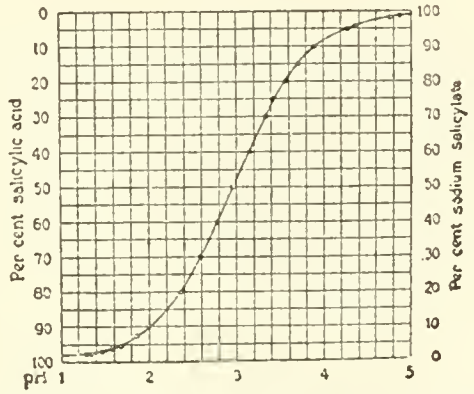


FIG. 2. LIBERATION OF SALICYLIC ACID FROM SODIUM SALICYLATE ACCORDING TO HYDROGEN ION CONCENTRATION (pH) OF THE SOLUTION AT 40°C.; AFTER BOOTS AND CULLEN (138)

ate, it is suggestive at least that salicylate might exert its beneficial action by virtue of the free salicylic acid in the inflamed tissues where the carbon dioxide tension may be very high. According to Ewald (136), the normal carbon dioxide tension of tissues is 6 per cent, but in inflamed tissues it may reach 20 per cent, and may be 5 to 7 per cent higher in inflammations than in asphyxias. Unfortunately, however, the tests of the acid theory that have been made have yielded only negative results. For instance, Hanzlik (137) found that definite liberation of free salicylic acid from sodium salicylate in pure buffer solutions required a hydrogen ion concentration equivalent to a pH of 6.5, and that the presence of 25 per cent serum or plasma protein required a much higher degree of acidity, namely a pH of 5.9, both being

degrees of acidity incompatible with, and indicating the improbability of demonstrating free salicylic acid in the blood during life. This was fully confirmed on animals subjected to fatal asphyxia and whose cardiac and arterial blood became slightly acid (pH 6.8). Extracts of salicyl-containing fluids from inflamed joints of rheumatic patients were found by Scott, Thoburn and Hanzlik (122) not to contain free salicylic acid. This might be expected, for joint fluids of rheumatic fever are probably usually alkaline, as indicated by the results of Boots and Cullen (138), who found values of from pH 7.27 to 7.42 for sixteen joint exudates. Consequently, the theory that free salicylic acid, liberated by virtue of the greater carbon dioxide content of inflamed tissues and their fluids seems untenable. The results discussed above dispose also of another theory, suggested by Poulsson (139), namely, that the absence of a right-sided valvular (tricuspid) endocarditis is due to the antiseptic qualities of free salicylic acid liberated here by virtue of the greater carbon dioxide content of the venous blood. The liberation of salicylic acid from sodium salicylate according to the hydrogen ion concentration (pH) of the solution at 40°C. is shown in figure 2.

Since the esters of salicylic acid are only partly hydrolyzed before absorption takes place, a considerable proportion of the unchanged compounds appears in the circulation and tissues (21, 23, 24). The acetylsalicylic acid appears as the sodium salt and possibly also the salicyl salicylate, but the methyl salicylate appears as such. This is probably the reason for the differences in pharmacological action and clinical toxicity of these compounds from those of ordinary salicylate. This is contrary to the current conceptions of the fate of acetylsalicylic acid, which was claimed by Gazert (140) among others to be decomposed almost entirely in the intestine, and its action, dependent on the liberated salicylate. The results of Hanzlik and Presho (21) on the decomposition of acetylsalicylic acid in alkaline and acid buffer solutions agree with those of Chistoni and Lapresa (141) who observed decomposition of the drug not only in alkaline juices, but also in gastric juice. These results are against the claims that acetylsalicylic acid is non-irritating in the stomach. Bondi and Katz (142) found that acetylsalicylic acid is absorbed in part as such from the intestine, though all is decomposed before excretion. These

observers also noted that strong trypsin preparations with the addition of bile decomposed this acid in five hours at 37°C. to the extent of 50 per cent; minced liver containing 1 per cent sodium bicarbonate accelerated the decomposition as compared with liver alone. Hanzlik and Presho (23, 24) observed no decomposing influence of bile and pancreatin on salicyl salicylate, and only some increase in decomposition of methyl salicylate on incubation with pancreatin. According to Fillippi (143), novaspirin and benzosalin are partly decomposed by living tissues and their ferments. However, benzosalin is expelled with the feces mostly unchanged.

Excretion and fate

General. Salicylates are excreted in the urine mainly as such, and apparently to a small, though variable, extent as salicyluric acid and a number of other products. On account of their rapid absorption, the feces, as a rule, contain exceedingly small amounts, if any at all. The presence of salicyl ethereal sulphate, salicylglycuronic and oxysalicylic acid in urine has been reported by C. Neuberg (144); ursalicylic acid by Baldoni (145). According to Nencki (146) saligenin (salicyl alcohol) in the body is converted into and appears as salicylic and salicyluric acids.

Sometimes the color of urine is rendered green after salicylate administration. Wolffberg (147) thought this to be due to a decomposition product of indican, which was increased. Fleischer (148) considered the greenish brown color to be due to alkapton formation rather than to change in indican or to pyrocatechin formation. Robin (149) regarded it as due merely to increased excretion of indican; G. Sée (150) as due to the formation of pyrocatechin from salicylate.

Urine containing salicylate reduces Fehling's solution. This was first observed by Fleischer (148); then by Smith-Pye (151) and later confirmed by Pollatschek (152).

The rate of excretion of salicylate varies with the dosage and diuresis. Drasche (62) found salicyl in the urine within a few minutes after administration of 0.01 gram by mouth; after 0.04 gram the urinary reaction was still present after 24 hours. After ordinary therapeutic doses the reaction did not disappear until at the end of five days. Large doses were necessary by subcutaneous injection to bring

about similar urinary reactions. Bälz (96) had the unusual opportunity to study the excretion in urine from the ureters of a patient with epispadias and defective anterior bladder wall so that catheters could be easily inserted into the exposed orifices of the ureters. After oral administration of sodium salicylate, the time of appearance of salicyl in urine was $8\frac{1}{2}$ minutes; of salicylic acid in 20 minutes, but unfortunately no statement is made as to the acidity of the urine, nor is the extraction method fully described. An increase in diuresis was also observed.

Duration of excretion. According to Geissler (153), complete elimination may take place in 12 hours; but ordinarily it lasts 24 to 48 hours, as observed by G. Sée. After doses of 1 to 2 grams of the salicylate, Blanchier (97) found excretion to be completed in 22 hours; after 4 to 5 grams at the end of 44 hours. In rheumatic individuals receiving 4 to 8 grams, traces were found in urine at the end of 72 hours. Herbivora excrete salicylate more rapidly than carnivora, as observed by Feser and Friedberger, quoted by Blanchier (97), and for this reason endure much larger doses. Blanchier stated that the time of appearance in urine is about 12 minutes after administration, in bile 30 minutes, and in saliva 45 minutes. Cornet (154) observed the appearance of salicyl in urine in $\frac{1}{2}$ to $\frac{3}{4}$ of an hour after administration of salol and the reaction was still present at the end of 46 hours. A diminution and prolongation in the excretion of salicylate has been observed in disease of the kidney and circulatory organs by Chelchowski (155) and Purpus (156). Brouardel (157) claimed the time of appearance in urine in older individuals was longer (2 hours) than in the young (15 minutes), and lasted longer in the old (2 to 8 days) than in the young (24 hours). However, Ehrmann (158) found excretion to last from 36 to 48 hours in normal individuals, and this was not different in rheumatism. The administration of alkali was thought to hasten the excretion. After the cutaneous application of salves, Schumacher (66) found the excretion to last 2 days. According to Herissey, Fiessinger and Debray (159), 0.002 gram sodium salicylate by mouth just gives a positive iron salicyl reaction in urine, and after a dose of 0.2 gram the reaction lasts 6 hours.

The excretion of salicyl in urine after the administration of acetylsalicylic acid and novaspirin was found by Block (160) to begin in

about ½ hour and last 2 days. It was also reported by Fillippi (108) that the excretion of salicyl in the synovia and urine of rabbits after administration of acetylsalicylic acid was slower than after sodium salicylate. After taking 1 gram of sodium acetylsalicylate, Dreser (11) reported that excretion of salicyl was completed at the end of 12 hours. The observations of Pinczower (80) indicated that the appearance of salicyl in urine after salicylic acid, sodium salicylate, acetylsalicylic acid, novaspirin, salol, salipyrin and benzosalin presented no striking differences. According to Rocco (161) excretion of salicyl salicylate lasted 22 hours with rabbits and 73 hours with dogs.

After full therapeutic doses and under controlled conditions, the differences between different salicyl compounds as to duration of excretion is more strikingly demonstrated. Hanzlik, Scott and Thoburn (162) and Hanzlik, Scott and Reycraft (163) observed a median duration of 78 hours in normal subjects, of 72 hours in rheumatic patients, and of 80 hours in patients with miscellaneous conditions. After acetylsalicylic acid in convalescent and rheumatic patients, Hanzlik and Presho (21) found the duration to be a median of 114 hours; after salicyl salicylate in convalescents, a median of 101 hours, and after doses of 1 gram each in 3 normal subjects, a median of 48 hours (23). With methyl salicylate in convalescents, the duration of excretion was a median of 96 hours, and after 1.18 gram doses in 3 normal subjects, 55 hours (24). In 4 dogs and 1 cat receiving a median dosage of 0.2 gram per kilo of methyl salicylate gastrically and intramuscularly, the median duration of salicyl excretion observed by Hanzlik and Wetzel (164) was 96 hours. It is obvious, therefore, that the salicyl derivatives require a much longer time for completion of excretion than sodium salicylate, despite the larger, absolute dosage of the latter. Even the smaller (1 gram) doses of the derivatives seem to indicate a tendency to retention. The tendency to longer retention in the body may be one factor among others in the greater toxicity and pharmacologic activity of certain of the derivatives. A summary of the results on duration of excretion of the different salicylates is presented in table 1, which contains the main results of our excretory studies.

Quantitative methods of studying excretion. Feser and Friedberger (131) were the first, in 1875, to study the quantitative excretion of

TABLE 1

Summary of urinary excretion of different salicylates in normal individuals, in rheumatic fever and other diseased conditons and in animals after full and small therapeutic doses

COMPOUND	NUMBER OF DIFFERENT INDIVIDUALS	DIAGNOSIS	MEDIAN TOTAL DOSE ADMINISTERED	MEDIAN DURATION OF EXCRETION	MEDIAN TOTAL SALICYL EXCRETED	MEDIAN TOTAL UNCHANGED DERIVATIVE EXCRETED (RANGE IN PARENTHESIS)
			grams	*hours*	*per cent*	*per cent*
Sodium salicylate	11	Normal	14.6	78*	79.7	
	8	Rheumatic fever	16.0	72†	60.4	
	6	Miscellaneous diseases‡	11.6	80	57.8	
	9	Animals (8 dogs and 1 cat)	1.57§		56.6	
Acetylsalicylic acid	4	Convalescents	7.0	117	80.0	22.7 (10.5–36.6)
	3	Rheumatic fever	12.0	114	68.5	33.4 (21.4–41.1)
Salicyl salicylate	4	Convalescents	8.5	101	63.0	6.2 (6.1–17.6)
	3	Normal	1.0	48	66.0	
Methyl salicylate	7	Convalescents	7.96	96	50.8	0.098** (0.055–0.116)
	3	Normal	1.18	55	50.5	
	5	Animals (4 dogs and 1 cat)	1.54††	96	25.9	0.36‡‡ (0.2–0.52)

* Accurate data from 6 subjects.

† Accurate data from 6 patients.

‡ Chronic nephritis, chronic alcoholism and morphinism, tuberculosis with fever, Basedow's disease.

§ 0.2 gram per kilo gastrically, intramuscularly and hypodermically.

** Approximate from 5 subjects.

†† 0.22 gram per kilo gastrically.

‡‡ 14.4 per cent after intramuscular injection in one animal.

salicyl in urine. The salicylic acid was recovered by extraction of the urine with ether. These authors recovered 63 per cent of the quantity administered, and drew the conclusion that the rest of the salicylic acid was destroyed in the organism, since it was not excreted by the feces. This was claimed to be incorrect by U. Mosso (165) because Feser and Friedberger did not consider the presence of salicyluric acid. Mosso claimed that salicyluric acid is only partly soluble in ordinary ether, but much more soluble in ethyl acetate; so that with a mixture of ether and ethyl acetate both the salicylic and salicyluric acids could be completely extracted. Since the results of Mosso have been frequently quoted without sufficient appreciation of the difficulties of the method used, a short description of the procedure will be given here.

In detail the quantitative separation, as carried out by Mosso, consists in first removing the mucoid and other substances by precipitation of the urine with neutral lead acetate; the precipitate is removed and washed salicyl-free. Then the filtrate is treated with ammonia and lead acetate and heated. The precipitate, which now contains the salicylates, is removed on a filter paper and decomposed with ammonium carbonate or sulphuric acid, filtered, and the precipitate washed until salicyl-free. From the filtrate the salicylic acids are now removed by repeated extraction with small quantities of a mixture of ether and ethyl acetate. This requires about 6 to 8 extractions. The ethers are allowed to evaporate spontaneously from a suitable dish and the crystalline residue remaining is weighed. The whole is then heated on a water-bath until the weight becomes constant; it is weighed again, and the weight of this second residue corresponds to salicyluric acid. The difference between the weights of the two residues corresponds to salicylic acid which had volatilized. Mosso collected urine for from 2 to 3 days, and recovered 96.8, 98.5, 102.1 and 106.7 per cent of the total salicylate administered. His conclusion was that salicylic acid is not decomposed in its passage through the body. It is not certain if Mosso made complete collections of urine or not. The application of Mosso's method by Thoburn and Hanzlik (166) to complete collections of three urines gave recoveries of 126.14, 215 and 223 per cent, and the salicyluric portions from eight animal and human urines were unobtainable. Hence, it was concluded that the method

was not suitable for quantitative estimations of salicyl. This was also the conclusion with respect to a number of other methods depending on extraction of salicyl by immiscible solvents directly from, or its direct colorimetric estimation in, urine.

The governing principle of a good method is the recovery of the total salicyl in pure form, or in aqueous solution free from any disturbing elements, before it can be estimated quantitatively. The estimation can then be easily and accurately made by the color reaction with iron (ferric). The method described by Thoburn and Hanzlik (166) meets all the requirements for urine and other body fluids. Applied to urine, it consists briefly of the hydrolysis of an aliquot portion of the specimen with 85 per cent phosphoric acid, distillation with the aid of steam until all the salicyl is driven off, and colorimetric estimation of the distillate with ferric alum. For details and its application to other body fluids the original should be consulted. The method has been used successfully by a number of investigators and applied by many trained and untrained workers to hundreds of urines and body fluids in our studies during the past ten years. The method has been frequently controlled in published and unpublished experiments with extraction methods in which different immiscible solvents were used and the residual urines treated in various ways for the detection of any unremoved salicyl. The acid residues left after the distillation of 20 different urinary specimens have been repeatedly shaken out with ether, ethyl acetate, chloroform and benzene, and in no single instance was there a positive iron test for salicyl obtained from such extracts. The agreement in the majority of instances has been as good as could be expected, and in many cases the quantities of salicyl recovered have checked nearly perfectly. This is well illustrated by the excretory results in the studies of Hanzlik and Presho (21, 23, 24) on acetylsalicylic acid, salicyl salicylate and methyl salicylate. In other words, the hydrolysis-distillation method recovers all the salicyl excreted in urine, the recent claim of Holmes (167) to the contrary notwithstanding. Holmes' results with his modification of the hydrolysis-distillation method are not altogether satisfactory. Furthermore, he has overlooked five other publications by Hanzlik and associates, besides the one criticized by him, dealing with excretion of salicyl under a variety of conditions, in which high and low

recoveries have been obtained. Recently again, as on many occasions in the past, some high urinary recoveries of 95 to 98 per cent of the salicyl administered have been obtained in this laboratory with the original distillation-hydrolysis method. This, however, is not invariably true, since the total quantity excreted varies with the individual and the condition. We may now consider the quantitative excretion of the different salicyl compounds. A convenient summary of the principal results is presented in table 1.

Excretion of salicyl by normal and rheumatic individuals. Salicyl is incompletely excreted, a part being destroyed in the tissues. The administration of daily doses of from 0.21 to 2 grams of salicylic acid for several days to 12 normal subjects resulted in a recovery of 46.8 per cent of total salicylates in the urine (Wiley (168)). Wiley considered the remainder to be changed to salicyluric acid or stored in the body; but it appears that the quantitative method used for the estimation of salicyl was faulty. The high recoveries of Mosso (165) were mentioned above, but the method used was also unsatisfactory. Baldoni (169), who claimed that the data of Mosso and others on elimination were discordant, recovered a total of about 86 per cent with his method depending on immiscible solvents. Using full therapeutic doses of sodium salicylate, that is, about 15 grams, Hanzlik, Scott and Thoburn (162) and Hanzlik and Wetzel (26) found that about 75 to 80 per cent of the total administered was excreted in urine. Since the loss could not be accounted for in sweat and feces or by retention, it was concluded that about 20 per cent of the salicyl was destroyed in its passage through the body. After administration of one gram doses to eight human subjects and making complete collections of urines, Devrient (170) recovered only from 1.01 to 14.68 per cent in urine and concluded that the remainder was destroyed or adsorbed in the body. He denies the claims of Mosso (165) and Bondzynski (82) that excretion is complete, and also the claim of Brouardel (157) that age affects excretion, since young and old subjects excreted about the same quantities in his experiments. With his sulphuric acid-hydrolysis-distillation method, Holmes (167) claims to recover usually over 90 per cent of the total (2 to 5 grams) sodium salicylate administered, but his lower recoveries of from 70 to 80 per cent are left unexplained. After taking 1.5 and 2 grams of salicyluric acid, about

84 and 85.7 per cent were recovered. On the whole, therefore, the percentage recoveries of this author, with the alleged improvement in method, are no better than those of previous investigators and the limited number of his experiments does not settle the question of destruction.

In rheumatic fever, Hanzlik, Scott and Thoburn (162) found the total excretion was only 60 per cent, that is, about 15 to 20 per cent less than in normal individuals. This difference was greatest in the early periods, i.e., during the first ten to twenty hours after administration of the drug. The concentration of salicyl in the blood and urine of rheumatic patients at "toxicity" was also less than in normal individuals. The differences were not due to diuresis, nor to retention and vicarious excretion of the salicyl. They seemed to be due to increased destruction of the salicyl in the febrile rheumatic organism.

Excretion in other fevers and disease conditions. A markedly diminished excretion was found by Hanzlik and Wetzel (26) in patients with febrile tuberculosis and syphilis and with Basedow's disease, in drug habitués (chronic alcoholism and morphinism) and in nephritis of both man and dog. The loss of salicyl, amounting to about 40 per cent, was attributed to increased capacity for destruction due to the increase in metabolism (katabolism) in the febrile conditions and Basedow's disease, and to retention with prolonged exposure to the destructive action of the tissues in nephritis.

Bicarbonate on excretion. Fleischer (148) claimed that the administration of sodium bicarbonate shortened the period of elimination of salicyl from 36 to 14 hours. Ehrmann (158) also claimed that alkali hastened the excretion, but Hanzlik, Scott and Thoburn (162) and Hanzlik, Scott and Reycraft (163) were unable to confirm these claims in patients receiving full therapeutic doses of sodium salicylate and doses of bicarbonate up to 16 grams. In the majority of patients the dosage of salicylate and bicarbonate was equal and the urines were definitely alkaline in all, but without demonstrable influence on the duration and percentage of excretion.

Excretion of esters and other derivatives. These are excreted partly unchanged and partly as ordinary salicylate, the total excretion being generally less than after the administration of sodium salicylate. Baas (84) found that the salicyl excreted after ethyl salicylate corre-

sponded only to 21 per cent of the ester given; with methyl salicylate only about 24 per cent. Baas concluded that these esters were only partially hydrolyzed in the intestine, a part remaining unabsorbed, and in part the esters were excreted combined with ethereal sulphates. Old urine possessed the odors of these esters, since their ethereal sulphate compounds had broken down and liberated the esters. Salicylamid was found to behave similarly, but salol was found to be entirely decomposed. After the application of 2 grains of methyl salicylate to the skin, Linnoisier and Lannois (68) found 10 per cent of the salicyl in the urine in 24 hours; after 4 grains, 25 to 30 per cent. After internal administration of ethyl salicylate, Bondzynski (82) claimed 91.3 per cent of salicyl in the urine; no ester was found in the feces.

Using full therapeutic doses, Hanzlik and Presho (21, 23, 24) made a systematic study of the urinary excretion of acetylsalicylic acid, salicyl salicylate and methyl salicylate. The results were checked by two quantitative methods, that is, by hydrolysis-distillation and by a special extraction technique in which the residual urines were treated for any unextracted salicyl so that all the salicyl reacting with iron, which includes salicyluric acid, was removed. From 4 to 14.8 grams of acetylsalicylic acid were administered to 6 subjects of whom 4 were convalescents and 2 suffered with rheumatic fever. The results of uncomplicated experiments were as follows: a total salicyl excretion of about 80 per cent in the convalescents and 68.5 per cent in the rheumatics, a difference that agreed quite well with that observed with sodium salicylate. From 8.8 to 36.6 per cent of the acetylsalicylic acid administered was excreted unchanged, and this was not influenced by the clinical condition, dosage of the drug, diuresis, total salicyl excreted and some other factors. The excretion of unchanged acetylsalicylic acid was anticipated from the results of hydrolysis experiments in vitro discussed in the section on absorption. After 2 gram doses in patients, Devrient (170) recovered 3.6 and 2.25 per cent of the unchanged acetylsalicylic acid in urine, and after 1 gram magnesium acetylsalicylate, 2.8 per cent. Using a method similar in principle to that of Hanzlik and Presho, Chistoni (171) recovered about 33 per cent of unchanged acetylsalicylic acid after 1 gram doses of the drug in human subjects.

Doses of from 4 to 12 grams of salicyl salicylate in 4 convalescent

subjects yielded a total salicyl excretion of 63 per cent, and of 66 per cent in 3 normal subjects, receiving 1 gram doses of the drug (Hanzlik and Presho (23)). In the 4 subjects receiving the high doses, about 6 per cent of the total salicyl salicylate administered appeared unchanged in the urine. This result was partly predicted from the hydrolysis experiments in vitro, since these suggested that the conditions in the alimentary tract would permit only a limited decomposition of the drug. The results on the recovery of total salicyl agreed with those of Baldoni (169) who recovered an average of 65 per cent in 3 subjects.

In 6 convalescent patients, who received from 6.58 to 10.72 grams of methyl salicylate, the excretion of total salicylate was rather variable, the median being 50.8 per cent, uninfluenced by dosage, diuresis and clinical condition. Three normal subjects receiving 1.18 grams each of the methyl salicylate showed a total salicyl excretion of about 50.5 per cent (median). The excretion of the ester in 3 patients was from 0.055 to 0.116 per cent of the total administered. This was much less than could be predicted from in vitro experiments on hydrolysis which was very small under the most favorable conditions. Hence, it seemed that the alkalinity, enzymes and bile of the alimentary tract did not participate much in the considerable liberation of sodium salicylate from the ester in its passage through the body. Apparently the greater part of the methyl salicylate was hydrolyzed in the tissues. The percentage excretion of methyl salicylate in human subjects was less than that found by Hanzlik and Wetzel (164) in animals which excreted from 0.2 to 0.52 per cent of the total administered (0.22 gram per kilo) gastrically. The greater variability of excretion of methyl salicylate as compared with salicyl salicylate and acetylsalicylic acid may be due to slower and irregular absorption suggested in the experiments. However, this does not account for the incompleteness of its excretion.

Although the excretion in feces was not studied with any of these derivatives, it appears improbable that expulsion with the feces, or incomplete absorption, would account for the losses, since the total excretions after small doses (around 1 gram) of salicyl salicylate and methyl salicylate were about the same as after the large or full therapeutic doses. Finally, complete collections of urines were made in all

cases, that is, until they were salicyl-free. Therefore, the loss of total salicyl after these derivatives, as also after sodium salicylate, seems best accounted for by destruction of the salicyl group in the tissues.

Excretion in animals. This is generally less after sodium salicylate, but more after methyl salicylate than in man, while after acetylsalicylic acid the excretion is about the same. Hanzlik and Wetzel (26) found the total excretion of sodium salicylate in 8 healthy dogs and 1 cat receiving about 0.2 gram per kilo gastrically, intramuscularly and hypodermically to be a median of 56.6 per cent, and this remained practically unaltered in 2 dogs whose livers were degenerated by treatment with phosphorus, and in 2 dogs and 1 cat receiving powdered thyroid with the idea of increasing the destruction. The excretion in normal animals, individually and collectively, was much less than in normal human subjects, and is indicative of a greater capacity for destruction. In a dog with experimental nephritis from salicylate itself, the total excretion was reduced to 26.6 per cent and this was in line with the small excretions of patients with nephritis. As for acetylsalicylic acid, Pitini (172) administered 1 and 1.5 gram doses to 6 dogs and recovered from 19 to 33 per cent of the total salicyl excreted as the unchanged drug, which is about the same as in the human subjects of Hanzlik and Presho (21). Methyl salicylate was mentioned above.

Destruction under biological conditions. The excretory results discussed above point to some destruction of the salicyl group in its passage through the body. There are other evidences which indicate the relative ease with which salicyl is decomposed by living organisms. It is a familiar fact, though formerly insufficiently appreciated, that solutions of sodium salicylate and other salicyl compounds gradually lose strength on standing. This is easily demonstrated in weak solutions of salicylate used as standards in quantitative estimations. Such solutions do not retain their original strength for more than about two weeks. The deterioration is due to some form of living matter such as fungi, since solutions containing a preservative (chloroform) and free from fungi do not deteriorate. This evidence was obtained in an extensive study of the destruction by Hanzlik and Wetzel (26), who observed, in addition, that destruction of the sali-

cylate occurred by yeast, though somewhat less than by the fungus growing naturally in salicylate solutions, and by hashed organs of animals. The destruction did not appear to be the special function of a particular organ, such as the liver, since the excretion in certain diseases of the liver in man and in hepatic degeneration in animals was within the normal range. Finally, the destruction was found to be greater in fever, Basedow's disease and nephritis, that is, during increased katabolism and in retention, than in normal subjects. It was also greater in animals than in man. Devrient (170) could recover only from 4.5 to 16 per cent from hashed organs, but some adsorption could not be eliminated, since the addition of salicylate to gelatin did not permit complete recovery, that is, at most 44 per cent. Taken as a whole, therefore, the evidence brought forward would seem to leave no doubt concerning the destruction of the salicyl group by living organisms. The destruction appears to be a part of the general function of metabolism. This view is contrary to the older views of Nencki (146) and Mosso (165) whose work, however, can not be accepted without question. It is consistent with the familiar chemical fact that salicylate when heated with alkalies is rather readily changed to phenol. The destruction of salicyl by living organisms should be of some practical interest in the metabolism of phenols and other aromatic derivatives. It may help to appreciate why salicyl in low concentrations is not an effective antiseptic in the blood and tissues; the necessity of using large doses to secure therapeutic effects; its harmlessness when used in low concentrations as food preservative, reference, of course, not being made to free salicylic acid and its local effects.

Salicyluric acid. The existence of this elusive product in the body has been denied and affirmed from time to time since the first report of its occurrence in urine by Bertagnini in 1856 (173). Bertagnini obtained a crystalline residue by ether extraction which, when exposed to a current of air at 140° to 150°C., yielded two products. That which volatilized was declared to be salicylic acid and the remaining nonvolatile portion, salicyluric acid, because when purified it possessed the following properties: melting point 160°C, it was readily soluble in ethyl acetate and alcohol, but less so in ether; more soluble in hot water, but almost insoluble in cold water; the taste was bitter, and it

possessed an acid reaction. The elemental composition, including nitrogen, answered the theoretical one for salicyluric acid and the empirical formula was given as $C_{21}H_{22}N_2O_9$. From the following formula usually given for it, $C_6H_4 \cdot OH \cdot CO - NH \cdot CH_2 \cdot COOH$, it is seen that it is a conjugation product of salicylic acid with glycocoll and, therefore, chemically analogous to hippuric acid ($C_6H_4 \cdot CO - NH \cdot - CH_2 \cdot COOH$). Its chemical properties resemble those of salicylic acid and it gives the characteristic pink to violet reaction with iron. Accordingly, any method depending on the iron reaction for the estimation of total salicyl directly in solution or extractives must include salicyluric acid. For over 60 years after Bertagnini, nearly all investigators followed essentially the same procedure, except for minor modifications, in the isolation of salicyluric acid as employed by Bertagnini, with variable successes. A brief résumé of these later researches follows mainly to illustrate their uncritical nature.

In a report by Gnehm (174) to the Swiss Chemical Society in 1875, it is stated that Piccard was able to confirm the results of Bertagnini, although he objected to the method of purification (by means of sublimation) recommended by Bertagnini as insufficient to give pure salicyluric acid. No data are presented, but according to Neubauer and Vogel (175), who quote Piccard, the separation of the two acids, i.e., salicylic and salicyluric acids, was accomplished by crystallization from ether and benzene, the salicylic acid crystallizing first. According to Stockman (176), salicyluric acid is insoluble in boiling benzene, and according to Baldoni (169), in chloroform. These solvents were used by these investigators to separate salicylic from salicyluric acid in urinary residues obtained by ethereal extraction. However, in neither case were the products sufficiently studied so as to identify them as salicyluric acid. In some earlier work, Baldoni (177) determined the melting point of salicyluric acid as 178°C. Stockman did not determine the melting point of his products, but identified them by their solubilities, by testing for ammonia (nitrogen) by soda lime and by their action on yeast and some common bacteria. Obviously such tests are not sufficiently specific and definite. According to Stockman, the urines after administration of salicin, saligenin and methyl salicylate contained salicyluric acid. U. Mosso (165) required the use of ethyl acetate besides ether for complete extraction of both

the salicyl acids and claimed to be able to separate salicyluric from salicylic acid in his quantitative method for the estimation of salicyl in urine, but Mosso also did not identify the supposed product as salicyluric acid. He concluded that most (up to 80 per cent) of the excreted salicyl in urine of man consisted of salicyluric acid, while in the dog the opposite was true. On the other hand, Baas (84) claimed that salicyluric acid can be completely extracted from acidified salicyl urine by ordinary ether alone. Using the method of Mosso, no salicyluric acid could be detected by Wiley (168) in the urines of human subjects taking salicylate. Nencki (146) claimed to have recovered salicyluric acid from urine by ether extraction of the acidified extractive obtained from urine treated with neutral lead acetate and then with alcohol. The melting point of his product was 159°C. and the elementary analysis for carbon, hydrogen and nitrogen corresponded to the theoretical, but it is not clear how the figure for nitrogen was obtained. Nencki stated that the decomposition of salicyluric acid into glycocoll occurs easily in urine. Using a method similar to that of Nencki, and after the administration of salol and *b*-naphthol salicylic acid, Lesnik (45) obtained a product which melted at 160°C., and, from the analysis of the carbon and hydrogen content, he calculated the nitrogen content (without actual determination), and these figures put together corresponded to the theoretical formula for salicyluric acid. However, Lesnik doubted if salicyluric acid was the only salicyl product in urine. In his earlier work, Baldoni (178) asserted that he could demonstrate salicyluric acid in the human, but not in dog urine. In dog urine, two other salicyl compounds were obtained, one containing nitrogen, i.e., uraminsalicylic acid ($C_{15}H_{16}NO_8$), and the other free of nitrogen, i.e., ursalicylic acid ($C_{15}H_{14}O_8$). In uraminsalicylic acid, Baldoni claimed that the nitrogen was not in the amino form, but he failed to mention his tests. Both of these salicyl compounds gave an azure color with ferric chloride which Baldoni employed as the chief differential point from salicylic acid which gave a violaceous color. Similar modifications of the iron salicyl color reaction may occur in the presence of impurities and probably have no significance. Most of the salicyl in dog's urine, however, appeared uncombined, and only a small fraction as the two compounds. In later work, Baldoni (179) withdrew his former claims about the isola-

tion of salicyl glycuronic acid from human urine, and he regarded Neuberg's new product to be the same as his ursalicylic acid. He attached no importance to the use of lead acetate in the isolation of salicyl compounds from urine. In still later work on human urine, Baldoni (169) claimed that the data on the excretion of salicyl and salicyluric acids reported by Mosso and others were discordant, and, with his own method, claimed that the proportion of excreted acids after the administration of 5 grams sodium salicylate was 2.12 of salicyluric to 1 of salicylic acid. However, there was no identification of the salicyluric acid and the separation of the two acids was assumed to be complete by means of solvents. Nevertheless, Baldoni concluded that the greater part of administered salicyl is eliminated as salicyluric acid and that individual variations exist, but whether the conjugation can be facilitated or interfered with remained unsolved. From all that has been said, the unsatisfactory nature of the earlier reports on salicyluric acid is quite obvious. The more recent investigations, now to be discussed, are more critical, but no more decisive as to the regular occurrence of salicyluric acid.

It is obvious that before a product may be accepted as salicyluric acid it must possess certain physical and chemical properties that are distinctive and different from salicylic acid with which it is closely associated. Essentially this resolves itself into a determination of the melting point, estimation of the nitrogen content, and, if nitrogen is present, it must be shown that it exists in the form of *a*-amino acid nitrogen. In addition, it is desirable to know the solubility in different solvents, since it is upon this that the separation of salicylic and salicyluric acids was based by many investigators. It is very essential that the products be pure, for, so far as nitrogen is concerned, this might be derived from the amino nitrogen of hippuric acid occurring normally in urine, or from other nitrogenous constituents. The lack of information on this point and the nitrogen content of the alleged salicyluric products in previous investigations is notorious. Depending on these criteria, Hanzlik (180) attempted to isolate salicyluric acid in the urines of 12 human subjects and of 8 dogs and 2 cats, receiving small, medium and full therapeutic doses of sodium salicylate. The methods of determining the melting point, solubility and nitrogen, of purification, etc., can not be considered in detail here but they were

of the usual standard kind. The test for *a*-amino nitrogen was applied for the first time to salicyl urines in this study. This was done colorimetrically with the triketone hydrate (ninhydrin) reagent of Abderhalden which demonstrates conclusively the presence of the amino group in the alpha position, and, therefore, should be positive, as it was, with synthetic salicyluric acid and with hippuric acid. Pure synthetic salicyluric acid was prepared by the method of Bondi (181) and this and hippuric acid were employed as controls. Synthetic salicyluric acid may also be prepared according to the method of E. Fischer (182). In addition, the methods of Bertagnini, U. Mosso, Baldoni, Stockman and Nencki and various modifications of these, and also other methods, were applied to the urines under various conditions, i.e., freshly voided, preserved, standing and decomposed, with and without the use of heat. Briefly, the urinary extractives were completely negative for salicyluric acid, since all the criteria defined above were not fulfilled. The products that were obtained were not well characterized, did not have distinctive properties and glycocoll was not demonstrable. Therefore, it was concluded that salicylates were probably not converted into salicyluric acid in the living organism, and that the products that had been previously interpreted as salicyluric acid were presumably more or less impure salicylic acid. However, the more recent attempts of Drzimal (183) and of Holmes (167) indicate that some variable amounts of salicyluric acid may be removed from human urines.

Drzimal (183) reports the isolation of 0.98 gram crude salicyluric acid equivalent to about 13 per cent of the total salicyl residue of 7.3 grams from the urine of one patient receiving 5 grams of sodium salicylate. In other words, about 87 per cent of the salicylate excreted was excreted as such. Moreover, the total salicyl recovered by Drzimal from the urine with the hydrolysis-distillation method of Thoburn and Hanzlik (166) was 6.2 grams, which agreed quite well with the total of 7.3 grams of impure extractive recovered gravimetrically and from which the small quantity of 0.98 gram of salicyluric acid was obtained. This shows that the hydrolysis-distillation method is capable of recovering all of the salicyl products, including salicyluric acid, contrary to the claims of Holmes (167). Drzimal's salicyluric product was identified qualitatively by the same criteria as employed by

Hanzlik. Unknown quantities of the same product in some urinary extractives, and identified by essentially the same criteria, are reported by Holmes (167). By a procedure that is indeed questionable for the quantitative estimation of any nitrogenous product, let alone salicyluric acid, Holmes (167) arrives at the conclusion that, for doses of sodium salicylate from 2 to 5 grams, the ratio of salicyluric to salicylic acid in urine is a constant at the value of 60 to 40 (1.5:1). This is quite different from the ratio claimed by Baldoni (169), namely, 2.12:1. However, the variability of these ratios is not surprising, since these investigators did not isolate, and conclusively prove they were dealing with, salicyluric acid in their quantitative experiments. Using Baldoni's method, Chistoni (171) claims that 79.89 and 73.37 per cent of the total urinary salicyl in two human subjects receiving 1 gram doses of acetylsalicylic acid were excreted as salicyluric acid. However, Chistoni did not isolate the product. Thus it appears from the most recent work that some salicyluric acid may appear in some urines after the administration of sodium salicylate, but that the occurrence in different species and its regularity and quantity are unsettled. It is not clear why some investigators have considered a conjugation of the compound with glycocoll an indispensable event, for salicylic acid, being a phenol as well as an acid, could conceivably conjugate with sulphuric acid with the excretion of ethereal sulphates. In fact, certain investigators have reported the excretion of ethereal sulphates, and of other salicyl products.

Behavior of salicyluric acid in the body. This can be studied with the synthetic product, which seems to be practically inert. Stockman (176) found that hypodermic injections in rabbits gave no symptoms, and it produced no beneficial effects in rheumatic fever. Stockman suggested that the administration of frequent and large doses of sodium salicylate in rheumatic fever is necessary because of its rapid excretion and rapid conversion into the inert salicyluric acid. Baldoni (184) reports that the acid is excreted unchanged and that hepatic lesions prevent its synthesis while kidney lesions probably favor it. However, the originals of his publications were not available. After 1.5 and 2 gram doses of salicyluric acid, Holmes (167) recovered 84 and 85.7 per cent in urine.

Other salicyl products in urine. It was mentioned in the beginning

and in other parts of this section that C. Neuberg (144) reported the presence of salicyl ethereal sulphate, salicylglycuronic acid and oxysalicylic acids, that Baas (84) found the methyl and ethyl salicylates combined with ethereal sulphates and that Baldoni (178) found the uraminsalicylic acid and ursalicylic acids. Holmes (167) denies the possibility of glycuronic acid existing in salicyl urines, and Ciamician and Ravenna (185) claim salicyl undergoes partial oxidation in the organism into compounds which are not easily extractable. A product that has been extracted from dog's urine by Angelico (186) is gensitinic acid ($C_7H_6O_4$) after the administration of sodium salicylate. It was recovered with ether after acidification of the syrupy residue with sulphuric acid; is crystalline with a melting point of 197° to 198°C., and at 210°C. decomposes into hydroquinone. Chemically it is claimed to be oxysalicylic acid-2,5. Angelico concludes that in dogs at least salicyluric acid is not formed.

Excretion of salicyl in sputum. Excretion of salicyl and antipyrine in the sputum of diseased lungs, but not of guaiacol and terpenes, is reported by F. Falk (187).

Excretion of cinchophen. Nothing definite is known about this. Dohrn (188) believed cinchophen gave rise to hydroxy cinchophen and a number of other pyridine derivatives. Skorczewski and Sohn (189) concentrated 30 liters of urine from a man receiving cinchophen, and after extraction of the concentrate with ether, obtained a product with a melting point of 200°C. which they claimed was 2-phenylquinoline-4-carboxylic acid, an oxidation product of cinchophen. Using the diazo reaction with urine after taking cinchophen, Greinert (190) found no change in the excretion of oxidation products and of residual nitrogen, but there was an increase in organic, inorganic and neutral sulphur. Recently, Scheunemann (191) has made a study of the urine of rabbits receiving cinchophen and reported the presence of five products; a red pigment of unknown constitution, 5,6-dioxyquinoline,8-oxyquinoline,6-oxyquinoline and 6-oxy-4-quinoline (?). His method consisted of extracting with ether the concentrated urine previously acidified with hydrochloric acid, washing the ether extract with alkali and then applying certain tests to the brown, thick residue left after removal of the ether by distillation. The residue was soluble in benzene, crystallized from amyl alcohol, gave a blue green color

with iron, and a red to reddish brown with Millon's reagent. These tests, it is claimed, excluded phenol and indicated dioxyquinoline, the *p*-oxyquinoline giving with ferric chloride a weak yellow color. Scheunemann quotes Donath to the effect that the latter claimed oxidation of cinchophen to a pyridine-carbonic acid in the organism.

The expulsion of neocinchophen with the feces has been studied by Barbour and Lozinsky (86) and was discussed in the section on absorption. As only about one-third of the dosage administered (1.5 and 9 grams) was claimed to be absorbed, the urinary excretion of any products must be small.

Effects on metabolism

Nitrogenous. This is generally increased. Lessened urea excretion was noted in diabetic individuals by Fürbringer in 1875 (53). Bohr (192) noted a marked increase in protein decomposition in dogs. Wolfsohn (193) observed in two animals a diminution followed by an increase in nitrogen excretion; in four animals there was from the start an increased urea excretion. The total nitrogen and uric acid in urine of patients taking 3 grams of sodium salicylate were found to be increased by Byasson (194). Chirone and Petrucci (195) observed in dogs and cats that prolonged administration of salicylate leads to emaciation and loss of body weight. The results of C. Virchow (196) confirm those of Bohr. Salomé (197) found that urea excretion was increased only after large doses of the salicylate. In Kumagawa's (78) experiments the increase in protein decomposition averaged from 10.6 to 13.4 per cent over the normal. This was compensated for by a loss during the after-periods. There was an increase in reducing substances in urine (about 60 per cent). The same was observed with salol. Jolin (198) reports no influence on metabolism by salicylates. No definite conclusions can be drawn from the work of Tauszk and Vas (199) because of the uncertainty of the diet and the unreliability of the quantitative methods used. Goodbody (200) studied the distribution of nitrogen in urine during periods of salicylate medication. As compared with the normal, the specific gravity of the urine was increased, and this was due to increased excretion of nitrogenous substances, particularly urea. Ammonia was also increased. There was no effect on protein absorption. In Wiley's (168) subjects there

was a slight inhibitory effect on nitrogen metabolism. Creatinine nitrogen was slightly increased; urea unaffected; and ammonia decreased. There was an increased quantity of metabolized nitrogen or tendency to increased digestibility and absorption of nitrogen ingested. The specific gravity of the urine during salicyl periods was increased; the quantity of solids was also increased, and persisted through the after-period. This suggested that the katabolic activities of the organs were increased. The conclusions, however, are not fully convincing.

Under controlled conditions of dietary, etc., and using modern analytical methods, Denis and Means (201) demonstrated considerable increases (up to about 40 per cent) in the excretion of total nitrogen in two normal subjects and one septic individual receiving up to 6.6 grams of sodium salicylate. There were also increases in phosphates and uric acid during the salicyl periods, but the increase in total nitrogen tended to persist after the administration of salicyl. Recently, Ohtani (202) observed that moderate amounts not only of salicylic acid, but also of the *m*- and *p*-hydroxybenzoic acids, increase the nitrogen excretion in rabbits. On the other hand, large amounts, particularly of salicyl, diminish the excretion. This may be due to diminished excretory activity of the kidneys, which are injured by large doses, causing nephritis. Ohtani states further that all of the compounds increase the reducible constituents in urine, and that the *m*- and *p*-hydroxybenzoic acids always increase the ethereal sulphates of urine, but not salicylic acid.

Uric acid. Owing to the fancied connection of uric acid with the etiology of acute articular rheumatism, the earlier literature contains several contributions on the effects of salicylates on the excretion of this purine. These are now chiefly of historical interest, but are remarkable for the uniformity of agreement on this phenomenon, which has been confirmed by the latest researches. As early as 1877, G. Sée (150) thought that salicylate increased the excretion of uric acid in gout. Blanchier (97) thought that salicylate increased uric acid excretion in normal as well as rheumatic individuals; urea excretion was also increased. Marrot (203) found uric acid excretion to be increased in individuals suffering with rheumatic fever; lessened in fever-free subjects. In observations on himself, Salomé (197) found

that only large doses of salicylate increased the uric acid excretion. In dogs, Kumagawa (78) found the excretion to be increased from 31 to 45.6 per cent. Schreiber and Waldvogel (204) reported a constant increase in uric acid excretion. The most marked increase in uric acid excretion occurred on the third day of salicylate medication in a subject studied by Herter and Smith (205). Siegert (206) found excretion to be lessened. Herringham and Davies (207) observed an increase in excretion, but were not satisfied that the presence of salicyl in urine did not interfere with uric acid determination. In diseased conditions, a decreased uric acid excretion was noted by Martineau (208) in typhoid fever; of uric acid, urea and phosphates by Bouchard (cit. Blanchier (97)). Unfortunately, many of these earlier observers took no account of the dietary régimes, though the estimations were frequently made in 24-hour collections of urinary specimens.

Later work, carried out by modern and more reliable methods, supported the views of the older investigators, namely, that salicylates increase the excretion of uric acid. The increased excretion observed by Bohland (209) was considered to be the result of increased uric acid production, and was ascribed to the concomitant leukocytosis. Hack (210) also observed an increased uric acid excretion after salicylate, and also ascribed this to leukocytosis; but, as no statement is made when the leukocyte counts were made, it is difficult to judge of any existing relation between the increase in uric acid and the leukocytosis. With subjects on a constant protein diet, sodium salicylate was found by Schreiber and Zaudy (211) and by Schreiber and Waldvogel (204) to cause a greater excretion of uric acid than salicylamide, and these increases bore no relation to leukocytosis, which was variable and often lower at the time of the greatest increase in uric acid excretion. Lewandowski (212) reported that benzoic and quinic acids, as well as salicylic acid, increased the excretion, but gave no metabolic controls. In dogs, which were kept on a constant diet and fluid intake, Singer (213) found that acetylsalicylic acid increased uric acid elimination and suggested that this was due to a simultaneous increase in leukocytes.

In experiments on himself in nitrogen and phosphate balance, Ulrici (214) confirmed the work of previous observers, and found

that uric acid excretion was increased by sodium salicylate from 40 to 50 per cent. Ulrici could draw no conclusions as to the effects on leukocytes, since the counts were too dissimilar and since increases occurred also when no salicylate was taken. According to Hall (215), the endogenous purines in urine are increased by the administration of sodium salicylate, and this increase does not arise from excessive cell destruction, but probably from diminished destruction of the uric acid already present. Wiley (168) concluded from observations on 12 subjects that uric acid was decreased, but the figures indicate practically no change in the excretion; yet there was an increase in leukocytes in the blood. Rockwood (216) found acetylsalicylic acid to increase the excretion of uric acid, and concluded this to be due to an inhibition of the uricolytic ferment rather than to destruction of nuclein, since there was no simultaneous increase in excretion of phosphoric acid. Approximately a 30 per cent increase in uric acid excretion lasting till the end of the second day was reported by Dreser (11) after 2.2 grams acetylsalicylic acid administered to a subject on a constant diet.

The following interesting features regarding the effects of salicylates on uric acid excretion have also been described: Stookey and Morriss (217) found that the liver, kidney, spleen and muscles of dogs given sodium salicylate (0.1 gram per kilo) increased the decomposition of uric acid as compared with the organs of untreated animals. The time of exposure was from 44 to 144 hours; the treatment of the dogs lasted from 5 to 10 days, and 9 to 65 grams of the organs were used. Waucomont (218) reported that sodium salicylate diminished the excretion of uric acid and xanthine compounds in chickens with experimental gout produced by feeding horse flesh over long periods of time. According to Pohl (219) allantoin excretion is not increased by salicyl, but Nagashima (220) claims that it is, and that this holds for cinchophen and the benzoic and quinic acids as well as for salicyl. Before going to the mechanism of the increase in uric acid excretion, the reports on cinchophen will be presented.

An increase in uric acid output after administration of cinchophen was originally observed by Nicolaier and Dohrn (28), and confirmed by nearly all later investigators among whom were Weintraud (221), Plehn (222), Retzlaff (223), Schittenhelm and Ullmann (224), Wie-

chowski and Bass (225), Zuelzer (226), Skorczewski and Sohn (227), and Kehrer (228). However, the explanations of the phenomenon were varied and none was satisfactory. Nicolaier and Dohrn thought the increase was due to a "toxic" production of uric acid, Skorczewski and Sohn to a diminished oxidation, and Kehrer to stimulation of the enzymatic conversion of purine bases to uric acid. Brugsch (229) claimed that cinchophen mobilized deposited urates and this idea is being exploited even today despite the untenability of the notion on the basis of well known physical and chemical properties of the urates. Starkenstein and Wiechowski (230) claimed that cinchophen depressed the purine metabolism as indicated by the reduced excretion of allantoin in animals and by the reduced excretion of uric acid during the after-period in man. This metabolic action was claimed to be inconstant, and in gout the greater elimination of uric acid excretion was accounted for on the basis of a greater amount of the metabolite available from deposits. In later observations, Starkenstein (231) claimed that the cinchophen caused only a temporary increase in uric acid excretion by healthy subjects on a purine-free diet without further increases after administration of the drug during the subsequent period of diminished excretion. He argued that the increased excretion could not be due to increased formation, since in animals there was no demonstrable increase in allantoin output under similar conditions. Furthermore cinchophen retarded the action of purine-forming enzymes in the liver, especially the xanthine-oxidase. In contrast with cinchophen, calcium chloride decreased the excretion of both uric acid and allantoin and retarded the activity of purine-forming enzymes, and radium emanations increased the output of uric acid in man and of allantoin in rabbits. Weintraud (221) advanced the view that the drug exerted a selective action on the kidney and through this action removed uric acid from the body. Retzlaff (223) concurred in this view.

The older explanations of the increased uric acid elimination varied according to the views concerning the nature of purine metabolism and of gout and rheumatism. Urinary analyses alone could not settle the question and the older quantitative methods based on isolation of the metabolite were inadequate. The few blood analyses reported were unreliable. There was also some confusion from the fact that the

total nitrogen of the urine was not always increased during the uric acid increase. According to modern work on the subject, salicyl increases the total nitrogen excretion. Nevertheless, so far as the mechanism of the increased uric acid excretion is concerned, this seems to be the same for both salicyl and cinchophen and is concerned with the kidney rather than with some obscure change in metabolism or mobilization of the urates in the tissues. Pietrulla (232) observed that large (6 grams daily) doses of salicylic acid increased the excretion of uric acid from 0.25 to 0.6 gram in normal subjects on a purine-free diet, and suggested that the point of attack was similar to that of cinchophen. However, it remained for Folin and Lyman (233) and Denis (234) to demonstrate conclusively that the mechanism of the increased excretion by these drugs is identical.

Mechanism of the increased uric acid excretion by cinchophen and salicyl. Using the colorimetric method of Folin and Denis (235) for uric acid in urine and blood, Folin and Lyman (233) demonstrated that single and repeated doses of 3 and 5 grams of cinchophen invariably increased the uric acid in urine and simultaneously decreased its content in the blood of 5 convalescent subjects and one patient suffering with gout. These investigators concluded that the increased output represented the elimination of uric acid which had previously accumulated in the blood, and that the previous accumulation represented a corresponding kidney inefficiency. The cinchophen caused a diminution of the non-protein nitrogen and urea whenever these were present in the blood in usual amounts. This was in accord with the ideas of Weintraud (236), who demonstrated diminution of "rest" nitrogen of the blood after cinchophen. Folin and Lyman's gouty patient responded the same way as the convalescents, and, since the uric acid content of his blood increased on withdrawal of the drug, they concluded with Weintraud (221) that the action of the drug was on the kidney. Following Folin and Lyman, McLester (237) and Steinitz (238) also demonstrated that cinchophen lowered the uric acid content of the blood. Definite evidence of the similarity of the increased uric acid excretion after salicyl to that after cinchophen was supplied by Denis in 1915 (234), who showed, that after doses of 7 grams and over of sodium salicylate administered to 13 subjects on a purin-free dietary and suffering with different disease conditions, there

was a definite increase in uric acid excretion in urine with a simultaneous decrease in the uric acid content of the blood. From this it was concluded that salicylate lowered the threshold of the kidney for uric acid, and this increase in renal permeability was thought to hold not only for uric acid but also for other waste products including the supposed toxins of rheumatic fever. However, the non-protein nitrogen of the blood was not always increased during the increased output of uric acid, and doses of 3.3 grams of the salicylate in some cases gave negligible changes in the uric acid. Nevertheless the results on uric acid agreed with those of Folin and Lyman (233) on cinchophen, and, hence, the mechanism of action of the two drugs was thought to be identical.

However, Frank and Pietrulla (239) feel that the last word on the subject of uric acid excretion by cinchophen has not been said, and they criticize the colorimetric method used by Folin and Lyman (233). There is no doubt that the uric acid reagent of Folin and Denis (235), which is also a reagent for phenols, can not differentiate between uric acid and salicyl and probably also the products after cinchophen. The increased values for uric acid, therefore, are due in part to these drugs and their products in the urine. This has been indicated by unpublished experiments in this laboratory, but the study has not been carried sufficiently far enough to show how far the results on uric acid excretion may require modification. However, it seems quite obvious that the salicyl, and also cinchophen, of the urine should be removed before the colorimetric reagent is applied. On the other hand, the lower figures for uric acid of the blood reported by Folin and Lyman, Denis and others are not consistent with an expected increase from the salicyl of the blood during the medication. The subject merits further study with another method. Ample confirmation of the difference between the uric acid content of the blood and urine by the colorimetric method after both salicyl and cinchophen, however, has been reported by several investigators.

Confirmation was reported by Fine and Chace (240), who administered cinchophen to patients, while radium bromide given intravenously or by inhalation of the emanations, did not affect the uric acid content of the blood. Later on, Fine and Chace (241) showed that sodium salicylate in from 3- to 6-gram daily doses and a 2-gram

dose of acetylsalicylic acid to 8 patients reduced the uric acid of the blood to from one-half to one-third of the initial content; the urinary excretion, however, was not studied, but a close resemblance of the action of salicyl to cinchophen was suspected. After the administration of repeated small and large daily doses (up to about 10 grams) of neocinchophen and cinchophen to 6 patients with different disease conditions, Myers, Killian and Simpson (242) and Myers and Killian (243) observed that not only uric acid but also urea and chlorides of the blood could be reduced by these drugs. It appeared also that arthritic patients, who were in the habit of taking these drugs and also salicylate, failed to respond with a lowering of the blood uric acid. Failure of reduction of uric acid after administration of cinchophen to 6 cases of nephritis was reported by Fine and Chace (244). However, Frank and Pietrulla (239) reported the usual effects on the blood uric acid in nephritic patients. In chronic leukemia, Levi (245) found cinchophen to augment the uric acid excretion in the same proportion as in health, but less than in gout; the purine bases and total nitrogen were also increased with appreciable change in the number of leucocytes. In agreement with this, Joël (246) found only a transitory increase in uric acid in leukemic patients and no action on leucocytes. As compared with cinchophen, equal doses of neocinchophen (0.4 and 0.5 gram 4 to 5 times daily) administered to 21 normal men on a purine-free diet were found by Haskins (247) to be somewhat less efficient in facilitating the excretion of endogenous uric acid. In iritis, Hawk and Smith (248) reported no increase in uric acid excretion after cinchophen though the drug was clinically beneficial, while neocinchophen increased the excretion.

Peculiarities of cinchophen on uric acid. Certain peculiarities in the action of cinchophen on uric acid under different conditions have been reported. Griesbach and Samson (249) observed some increase in uric acid of the blood during the first hour or two after administration of cinchophen followed by a gradual decrease which lasted from 8 to 12 hours. In experiments on uric acid injected intravenously with and without cinchophen, Griesbach (250) found that 15 minutes after injection of uric acid alone only a small part could be found in the blood, the remainder being absorbed by the tissues or destroyed, but after the administration of cinchophen, the absorption of the injected

uric acid was retarded and the absorbed amounts could not be recovered quantitatively. Against uricolysis was the fact that more uric acid was excreted than was injected. It was suggested that uric acid formation occurred under the stimulus of the injection. According to Graham (251), the source of the increased uric acid of the urine can not be the blood, since the total amount is too small, and the evidence that uric acid deposited in the tissues can be redissolved is too weak. Using the colorimetric method of Folin and Denis with blood and the Folin-Shaffer method for urine, A. Wolff (252) reported recently that intravenous injections of sodium cinchophen, in doses of from 0.5 to 2 grams used as a 10 per cent solution, did not reduce the blood uric acid of 7 starved patients suffering with bronchitis, plumbism, arteriosclerosis, arthritis, chronic nephritis and hydronephrosis. The uric acid of the urine was increased. The absence of change in blood uric acid, he thinks, argues against the tissue mobilization theory, and whether the increase in urine is due to an action on the kidney or nervous system is unsolved. As far as the blood uric acid results of Wolff are concerned, the failure to demonstrate a decrease may be due to the use of the colorimetric reagent reacting with the cinchophen, or its products in the blood, thus giving false values for uric acid. Rosenfeld (253) conceives of cinchophen, glycerine and alcohol acting the same way on uric acid, that is, by oxidation of exogenous and endogenous purines, but this seems rather speculative. An increase in the output of nucleic acid in the urine of normal individuals continuing more than three days after the administration of cinchophen is reported by Grabfield and Pratt (254). The increase exceeded the endogenous level. These observers report that cinchophen does not invariably "sweep out" the uric acid from the body of normal individuals. The oxidation product appearing in urine after administration of cinchophen to man, namely, hydroxyphenylquinoline carboxylic acid, is reported by Rotter (255) to increase the elimination of uric acid just like cinchophen itself. According to Borak (256), marked excretion of uric acid takes place also after irradiation of the liver and spleen by x-rays, and comparing this with the increases after administering cinchophen and drinking large quantities of water, he concludes that the mechanism of the excretion of these agents is similar, namely, a washing out of purines from the tissues. However, this does not follow from the mere similarity of effects.

Recently, Goldwasser (257) has observed that the amount of uric acid changes in surface tension in a 24-hour urine were more or less parallel after administration of cinchophen. The factor $E = (a - b) \cdot Q/100$ (in which Q represented the volume of urine, a, drops of urine and b, drops of water, by stalagmometer) and the amount of uric acid increased simultaneously on the addition of meat to a purine-free diet, but after the administration of cinchophen, E decreased although the uric acid increased. Recently, Stern (258) studied the comparative solubility of uric acid in salicylate and cinchophen. The solubility in 1 per cent sodium salicylate was double that in water, and trebled in sodium cinchophen. The increased solubility in salicylate was not due to the formation of sodium urate, since salicylic acid is a stronger acid than uric acid, and, therefore, it was suggested that the increase was due to some hydrotropic effect. In the case of cinchophen, the cause of the increased solubility was unknown, since the dissociation constant of cinchophen is unknown. Confirmation under controlled conditions (pH, etc.) is desirable before the results can be of significance in the prevention of uric acid deposits in urine. As for urate deposits in tissues, such experiments should at least be made with sodium acid urate in serum under the proper conditions of pH, carbon dioxide tension and temperature to be of physiological significance.

Sulphur. An increased excretion of sulphur in the urine, chiefly as sulphate (SO_4), was observed by Baumann and Herter (259). An increase amounting to from 10 to 19.6 per cent above normal was reported by Kumagawa (78). This was compensated for by a loss during the after-periods. The normal constant relationship between total nitrogen and sulphur became irregular during salicyl medication. Ethereal sulphates were found to be uninfluenced by Baumann and Herter (259), Moreigne (260) and Singer (213). Moreigne found the total sulphur, urochrome and phosphates increased. Wiley (168) found the relative percentages of ethereal and inorganic sulphates to be unaffected; only the relative proportions of neutral sulphur appeared to be increased. Combinations of salicyl with ethereal sulphates were mentioned in the fore-part of this section in conjunction with other salicyl products in urine.

Glycosuria and glycuronic acid. In connection with the discussion

of general excretion, it was indicated that at least three of the older reporters claimed reduction of Fehling's solution by salicyl urines. More recently, Kramer (261) reports that Trommer's test was positive with urines of a large number of individuals receiving acetylsalicylic acid. However, reduction of Fehling's solution is denied by Holmes (167), who contends that the absence of the sugar reaction is proof against the occurrence of glycuronic acid. No salicyl glycuronic compound was found with color tests. The fermentation test might not be satisfactory in the solution of the question owing to the possibility of some antiseptic action of salicyl urines, especially the highly concentrated ones after full therapeutic doses of the drug.

Reaction of urine. The acidity of urine is increased during salicylate medication according to Moreigne (260), Schreuder (262) and Jordan (56). No effect on the reaction was observed by Wiley (168).

Respiratory metabolism. Singer (213) claimed an increase in the oxygen consumption of rabbits after toxic doses of acetylsalicylic acid. However, Denis and Means (201) observed no changes in the respiratory quotient of 2 normal individuals and 1 septic patient receiving doses of sodium salicylate up to 6.6 grams. The spirometer type of apparatus was used under controlled conditions of dietary, etc.

Autolysis

This was observed by Lacquer (263) to be increased in muscles with weaker solutions of sodium salicylate (0.1 per cent); inhibited by stronger solutions (1 per cent). Dogs poisoned with large doses of sodium salicylate exhibited no increase in soluble nitrogen in fresh organs (liver and muscle), and there was no special rapid autolysis of these organs.

Blood

Reaction and acidosis. The symptoms of salicylism have been attributed by some to acidosis. Also the administration of bicarbonate, which is commonly given with salicylate, has been justified on this basis, i.e., to prevent the occurrence of acidosis. However, it is hardly conceivable that the true reaction (hydrogen ion concentration) of the blood could be sufficiently altered by salicylate so as to cause real acidity, and the results of old and recent observations con-

firm this opinion. As early as 1883, H. H. Meyer (264) showed that sodium salicylate could inhibit oxidation. but it did not influence the alkalinity of the blood. Walter (265) claimed that death in fatal poisoning from salicylates does not result from the production of acidity, since the blood of animals so poisoned contained insufficient (only 11.2 per cent by volume) carbon dioxide.

Using the colorimetric-dialysis method of Levy, Rowntree and Marriott (266) for pH, and the colorimetric-dialysis-aeration method of Marriott (267) for alkali reserve, of the venous blood of seven rheumatic and non-rheumatic individuals receiving full therapeutic doses of sodium salicylate to the point of salicylism, Scott, Thoburn and Hanzlik (122) found no noteworthy changes in the reaction and reserve alkalinity of the blood at "toxicity." The same was found to be true of 2 dogs and 2 cats treated with corresponding and larger doses of the salicylate. Moreover, the administration of approximately equal doses of bicarbonate and salicylate to 6 convalescents by Hanzlik, Scott and Thoburn (162) and Hanzlik, Scott and Reycraft (163) did not alter the "toxic" dose or prevent the symptoms of salicylism. This disproves the claim of Lees (268) that the administration of bicarbonate with salicylate modifies and prevents the symptoms of salicylism, and even convulsions. It is also against the claim of Delore (269) that bicarbonate prevents the side actions of salicylate. As far as systemic effects are concerned, Meara (270) is probably correct in his belief that the use of alkalies together with salicylates has been dictated more by tradition than by any rationale.

Salicyl and enol acids. It is interesting to note, however, that salicyl causes considerable acceleration of respiration with symptoms of air hunger just like certain enol acids occurring in diabetic acidosis. A state of ketosis appears to have occurred in each of 3 cases of poisoning from methyl salicylate reported by Rosenbloom (271), H. B. Myers (272) and Wetzel and Nourse (273). In all these cases there was some resemblance to diabetic coma and all 3 urines contained acetone, and 1 urine, diacetic acid. Moreover, Hurtley and Trevan (274) have reported that the subcutaneous and intravenous injections of sodium salicylate, ethyl acetoacetate, sodium acetoacetate and acetyl acetone in animals caused the phenomena of diabetic coma. None of these compounds caused changes in the pH of the blood, but all caused air

hunger and coma with increased pulse rate and final respiratory failure. The results agreed with the hypothesis that the phenomena of diabetic coma are due to poisoning by aceto-acetic acid in the form of salts and not to true acidosis. The enolic group, $-C\cdot OH = CH-$, which occurs in one form of ethyl acetoacetate and gives a color reaction with iron, is claimed to be 18 times as active as *B*-oxybutyric acid. Bearing in mind that salicyl also gives a color reaction with iron and markedly accelerates respiration, there may be possibilities in this interesting physiological conception in explaining certain features not only of the actions of salicyl but also of respiration itself.

Erythrocytes. The direct application of salicylic acid to red blood corpuscles tends to cause disintegration. Cotton (275) observed that a 3 per cent solution caused them to become globular and the hemoglobin was decomposed with the formation of hematin. Thiersch (276) noted that the central portion of the corpuscles became transparent and enclosed by a peripheral zone of yellow that broke up into segments which floated in the plasma. Swelling and discoloration of frog erythrocytes was observed by Prudden (277) with solutions of 1:1000 salicylic acid, and decolorization with destruction by solutions of 1:500. Human erythrocytes swelled and decolorized with concentrations of from 1:4000 to 1:1000, and became markedly degenerated with 1:500. After internal administration of salicylates, Marrot (203) saw a diminution in number of red blood-corpuscles. On the other hand, a slight increase in the number of both white and red corpuscles was observed in the metabolism experiments of Wiley (168).

Leukocytes. With dilute solutions of salicylic acid, Binz (278) observed a paralytic action on white blood-corpuscles, similar to that produced by quinine, and death with concentrated solutions. Cotton (275) found that a 1 per cent solution of salicylic acid caused the central portion of leukocytes to become transparent, and gave them a double contour. Prideaux (54) observed the movements of leukocytes to be depressed by salicylic acid, but the red-corpuscles remained unaffected. Prudden (277) obtained results as follows with the leukocytes of frogs, rabbits and man: With frogs, 1:4000 of salicylic acid in 0.5 per cent sodium chloride solution checked the emigration of leukocytes in the bladder and mesentery without alteration of the caliber of the vessels; the structure of the cells remained unaffected, but

ameboid motion on a warm slide was retarded; 1:1000 solution caused stasis in blood-vessels, and death of the leukocytes; 1:500 solutions caused instantaneous stasis and death of leukocytes with destruction of the protoplasm. With mammalian blood (man and rabbit), 1:4000 to 1:1000 solutions of salicylic acid inhibited the ameboid motion of leukocytes with death of the cells and disintegration of protoplasm; 1:500 caused immediate death of leukocytes with marked degenerative changes. In general, with either blood (amphibian or mammalian), the changes produced by salicylic acid are less than those produced by phenol; and the arrested ameboid motion cannot be rehabilitated, which resembles the effects of other dilute acids, such as hydrochloric and acetic.

Leukocytes in leukemia, etc. The use of cinchophen in leukemia was suggested by B. Mendel (279) on the basis of a leukopenia caused by 1 gram of the drug in a patient with gonorrheal arthritis, and of diminished ameboid motion and diapedesis of leukocytes in the mesentery of frogs injected with 5 mgm. of sodium cinchophen. Beneficial effects in various infections were reported by Mendel after intravenous injection of a mixture of hexamethyleneamine and cinchophen ("leukotropin"). In his study of the migration of leukocytes in the vessels of frog's mesentery, Ikeda (280) observed that much higher doses of cinchophen than of quinine were necessary to affect the cells. From 6.25 to 12.5 mgm. per frog were just effective, and it took 75 mgm. to completely inhibit the migration without depressing the circulation. When quinine and cinchophen were combined in one-third their maximum ineffective doses, they acted more forcefully than much larger doses of quinine alone. However, the leukopenic action of the drug is not universally accepted. After ordinary doses of 1.2 and 1.5 grams of the drug, Dohrn (281) found the leukocytes unchanged. Starkenstein (282) has criticized the conclusions of B. Mendel, because the latter injected hexamethyleneamine together with the cinchophen and he points out that the hexamethyleneamine was responsible for the destruction of the leukocytes, though the cinchophen may cause temporary paralysis of these cells. Using a mixture containing hexamethyleneamine, 1.5 grams, and cinchophen, 0.5 gram in 5 cc., Ullmann (283) claims that the leukopenia he observed was due to "vagus shock," but this interpretation seems doubtful. On the other

hand, Joël (246) and Levi (245) observed no appreciable change in the leukocyte counts of patients with leukemia, the condition in which the drug is alleged to be beneficial.

Depression of the leukocytes in rheumatic fever after administration of sodium salicylate, acetylsalicylic acid, neocinchophen and the ethyl ester of cinchophen has been reported recently by Swift, Miller and Boots (284). All of these drugs acted in essentially the same manner. The leukocytes fell sometimes to normal, and, on withdrawal of the drugs, they increased to 4000 or more above the previous level (persisting leukocytosis) indicating that the infection had not stopped. However, when the leukocyte curve remained normal on discontinuance of the drug, no relapse occurred and the patient recovered. The body weight of some of the patients increased during the medication, which suggests a possible retention of fluids in the body (hydremic plethora), and which in turn might explain the reduction in leukocytes owing to blood dilution. The mechanism of the depression of leukocytosis is not understood, but Swift, Miller and Boots suggest a direct effect of the drugs on the hematopoietic system or suppression of exudative inflammation of the arthritis which may be stimulating the leukocytosis.

Opsonins. Jacoby and Schütze (285) tested the question of opsonic function of serum in rabbits, and found that salicylic acid did not influence the process.

Gastro-intestinal tract

Local effects. The effects of the administration of free salicylic acid are largely local. The irritation leads to nausea (Moore (286)); vomiting and diarrhea, and in large doses, ulceration according to Deseille (287) and Weber (288). Benjamin (289) reported the presence of ulcers and erosion of the esophagus in a horse after the administration of large doses of salicylic acid. The effects of small doses may be avoided by the use of sodium salicylate. Bicarbonate is frequently prescribed with sodium salicylate to prevent the precipitation of free salicylic acid and thereby lessen the local irritation, but this does not prevent the emesis. Most salicylates possess a sweetish nauseating taste. Acetylsalicylic acid is acid, and Bercke (99) claims that the calcium salt forms a reliable neutral and tasteless salt.

Salicyl salicylate is practically tasteless, and methyl salicylate has the burning, disagreeable taste of volatile oils. The liberation of free salicylic acid from the esters in the alimentary tract has been discussed in the section on absorption; and other aspects of local action have been pointed out in the section on local action and irritation.

Cinchophen, being an acid, also causes local irritation and considerable gastric distress, but not so neocinchophen, which is an ester and less soluble. Gastric irritation and anorexia from cinchophen were observed by Heller (290) and this was fully confirmed by Hanzlik, Scott, Weidenthal and Fetterman (291) in a study of 17 human subjects receiving variable doses of the drug. These symptoms were generally absent after large doses of cinchophen in 10 other subjects. Absence of gastric distress after doses up to 16 grams of neocinchophen was reported by Barbour, Lozinsky and Clements (292), a similar claim being made also by Chace, Myers and Killian (293). However, Miller and Boots (294) observed quite as much abdominal distress after neocinchophen as after salicylates. In a later study, Miller and Boots (295) reported that these symptoms were milder in degree after the administration of the ethyl ester of cinchophen, which differs chemically from neocinchophen by the absence of the methyl group.

Emetic action. This occurs with all the salicyl and cinchophen compounds providing sufficient dosage is administered. The cause is unquestionably central with salicyl, and presumably also with cinchophen. In 1879, Blanchier (97) observed that sodium salicylate caused vomiting whether given gastrically or intravenously. In his study of the toxic and fatal doses of different salicyl compounds, Waddell (296) found animals to vomit quite as readily after intravenous as after oral administrations, and the salicyl was not found in the vomitus after hypodermic administration. But it remained for Eggleston and Hatcher (297) to demonstrate conclusively that the seat of the emetic action is central. This was done on three eviscerated dogs which responded to doses of 0.2 and 0.3 gram of sodium salicylate intravenously with nausea in $1\frac{1}{2}$ minutes and with vomiting movements in from $4\frac{1}{2}$ to 10 minutes after injection. Oral administration resulted in delayed vomiting and the dosage required was three times that intravenously, or, in other words, emesis did not occur from the local action, but only after adequate absorption and systemic action of the drug.

That the various derivatives (esters, etc.) of salicyl and cinchophen cause nausea and emesis in human subjects is readily seen from the reports of the following investigators: Hanzlik (298), Hanzlik, Scott and Thoburn (162) and Hanzlik, Scott and Reycraft (163) on sodium salicylate; Hanzlik and Presho (21, 23, 24) on acetylsalicylic acid, salicyl salicylate and methyl salicylate; Hanzlik, Scott, Weidenthal and Fetterman (291), Miller and Boots (294, 295), Chace, Myers and Killian (293), and Heller (290) on cinchophen, neocinchophen and the ethyl ester of cinchophen. Barbour and Lozinsky (299) did not observe emesis with the highest doses of neocinchophen in dogs, while doses of 0.5 gram of cinchophen and of 0.1 and 0.2 gram of acetylsalicylic acid caused emesis. Nausea and vomiting are also among the symptoms of poisoning reported by several to be discussed later in the section on toxicity. From the fact that the salicyl derivatives circulate through the tissues unchanged to a considerable extent (discussed in the section on excretion), it is indicated that they were mainly responsible for the nausea and emesis which occurred in the same subjects. As far as salicyl salicylate ("salysal;" "diplosal") is concerned, this is contrary to the claim of Minkowski (300), and liberation of salicylic acid does not occur in the stomach as claimed by Tocco-Tocco (301) and proved by the work of Hanzlik and Presho (23).

Secretions. The secretions of the accessory glands of the alimentary canal are increased at first, later diminished, according to Blanchier (97), who investigated this in dogs. In 4 to 5 minutes after intravenous injection of about 0.3 gram per kilo of the sodium salicylate, the increase in saliva became noticeable, which gradually increased up to 5 times above normal and continued for some time. After increasing the dosage, the secretion stopped almost entirely. The same sequence of phenomena was noted with bile. Pancreatic juice remained practically unaffected. Blanchier regarded the hypersecretion of saliva as due to a central action on secretory nerves, and the inhibiting action as due to a local action on the glandular elements in part, and partly to a paralyzing action on the nervous system. Hypersecretion of bile and saliva were also noted after gastric administration, although occurring somewhat later. In Wiley's subjects (168) there was a diminution of the total quantity and the water content of the feces. There was no tendency to produce diarrhea. The

dosage ranged from 0.2 to 2 grams per day, and about 30 grams were administered in thirty days. A marked increase in gastric secretion has been observed by Leichentritt (302) after administration of sodium salicylate, acetylsalicylic acid and salol to dogs with duodenal fistulae and on a diet of flesh and milk. The control secretion was from 296 to 365 cc.; after acetylsalicylic acid, 420 to 457 cc. The action resembled that of arsenic compounds.

Cholagogue action. The reports on this are contradictory, diametrically opposite results being reported with the same experimental methods. This is not surprising, since the operative procedures commonly employed in experiments for the purpose are apt to disturb the functions of the liver and the biliary passages, and this seems to be true of both acute and chronic experiments. The older investigators generally recorded increases in the secretion of bile after salicyl. Blanchier (97), Stadelmann (303) and Mandelstamm (304) found increases in dogs after sodium salicylate; and Pfaff and Balch (305), after salol. Urobilin in urine after salicylate was reported by Dominikiewicz (306). Petrowa (307) demonstrated, in dogs with gall-bladder and gastric fistulae and ligated ductus choledochus, that sodium salicylate increased the secretion of bile, and this was generally true of those compounds that are conjugated with ethereal sulphates. On the other hand, Smyth and Whipple (308) claim no effect from sodium salicylate, in therapeutic doses. on the excretion of bile salts in dogs with permanent biliary fistulae. Negative results also have been reported recently by Stransky (309) after doses of 0.1 and 0.2 gram sodium salicylate intravenously and intraduodenally in rabbits. These were acute experiments in which the cystic duct was ligated, and the bile and its solids measured after collection from a cannula in the ductus choledochus. Stransky states that his negative results agreed with those of Pohl and of Brugsch and Horsters on dogs with fistulae. In guinea pigs receiving methyl salicylate orally and hypodermically, Leone (310) observed a marked increase in the total amount of bile secreted, but the percentage of dry matter and ash was decreased. The viscosity and surface tension of the bile were diminished, while the osmotic pressure and electrical conductivity were increased, all of these changes being directly proportional to the amount of the ester administered. With the larger doses by mouth, the ester was

excreted into the bile. In another series of experiments with guinea pigs on a constant diet, Leone (311) confirmed that hypodermic injection of methyl salicylate increased the amount of bile secreted, but the total solid residue was also increased. Apparently the increase in secretion was out of proportion after doses of 1 gram as compared with doses of 0.25 gram.

The results with cinchophen are no more satisfactory than those with salicyl. In their studies with gall bladder fistulae, Brugsch and Horsters (312) found that intravenous, intramuscular and oral administrations of sodium cinchophen in doses of from 0.8 to 3 grams caused increases in secretion of bile from 8 to 864 per cent. The total quantity, solids, acids and pigment of 24-hour collections of bile were all increased; likewise the viscosity and surface tension quotients. The viscosity quotient for normal bile was between 2.4 and 11, and administration of cinchophen changed this to 16, 20 and 39. The alkalinity of some biles was increased, that is, the pH determined electrometrically increased from 7.4 to 7.98 before, to 8.3 after, cinchophen. A considerable increase in secretion of bile was produced in atropinized dogs, and therefore the augmentor action of cinchophen was not concerned with the autonomic nervous system. It was thought to be due to direct stimulation of the liver cell. Since quinine, optochin and carbohydrates also possessed a cholagogue action, there was nothing peculiar to or specific about cinchophen. Further confirmation of the action of cinchophen is reported by Horsters (313), who attributes it to an effect on the liver cells and attempts to reconcile it with the increase in uric acid excretion produced by the drug. However, Stransky (308) reports he was unable to confirm the work of Brugsch and Horsters. Using doses of from 0.05 to 0.2 gram of sodium cinchophen in 5 per cent concentration intravenously and intraduodenally in urethanized and unanesthetized rabbits, the secretion of bile was unchanged or diminished. Ether extraction of the acidified bile failed to reveal the presence of cinchophen in the secretion.

Clinically, intravenous and intramuscular injections of cinchophen in healthy subjects and patients with catarrhal jaundice are reported by Grunenberg and Ullmann (313) to have caused increases in the bile (ash and pigment) of duodenal contents and in the bilirubin content of serum. Unfortunately, the dosage is not stated, and in some cases

proprietary products were used one of which was a mixture of salicylate and cinchophen. Using the same method, i.e., the duodenal tube in patients, Teschenberg and Hoffmann (314) claim confirmation of the stimulating action of cinchophen on bile; magnesium sulphate, oil of peppermint and sodium glycocholate acted similarly. Slobosiano and Herscovici (315) report that daily doses of 0.1 gram of cinchophen together with 2 grams of sugar by mouth reduced the bilirubin content of the blood of 6 jaundiced infants, but taken without sugar the drug was vomited and no change in the blood was observed. The drug was also not retained when given in 0.1 gram doses twice daily per rectum.

Salicyl as a test of liver function. The use of salicyl for this purpose is uncritical, for the liver is not the sole organ in which transformation or destruction of salicyl may take place. In dogs with hepatic degeneration from phosphorus, Hanzlik and Wetzel (26) found the urinary excretion of salicyl to be quite as prompt and as good as in normal control dogs; and hashed liver caused no more destruction of salicyl than other organs. Therefore, these results did not support the statement of Dixon (317) that salicyl is chemically changed by the liver.

Moreover, the clinical reports on the use of salicyl as a test of liver function are distinctly uncritical. Roch (318) claims that the normal liver transforms 0.04 gram sodium salicylate taken one hour after breakfast, so that none can be found in urine during the next 5 hours, while, in hepatic insufficiency, a positive test with ferric chloride is obtained within this time. Roch claims further that salicyl induces formation of glycuronic acid in the liver, and that he has obtained reliable results in 100 patients. The claim as to glycuronic acid is unsupported by evidence, and as for the test of liver function, which depends on urinary excretion of salicyl, this is uncritical without quantitative estimation, and consideration of diuresis and factors influencing absorption, destruction, etc. Herissey, Fiessinger and Debray (319) claim that 0.2 gram sodium salicylate gives in normal subjects a urinary reaction with iron lasting about 6 hours, but, in cases of cirrhosis, the reaction is not so well marked. This is attributed by these authors to mechanical conditions resulting from portal stasis, etc., but, of course, under these conditions the intestinal absorp-

tion would be impaired and excretion diminished. Without estimations under controlled conditions, the test would have no meaning anyway.

Cinchophen and salicyl on excised intestine. According to Starkenstein (320), direct application of sodium cinchophen to segments of rabbit intestine causes depression and paralysis. With excised loops of rabbit and guinea-pig intestine, used according to the Trendelenburg method, Starkenstein (321) found sodium cinchophen (0.4 cc., 10 per cent) and "leukotropin" (0.3 cc., 40 per cent of a mixture con-

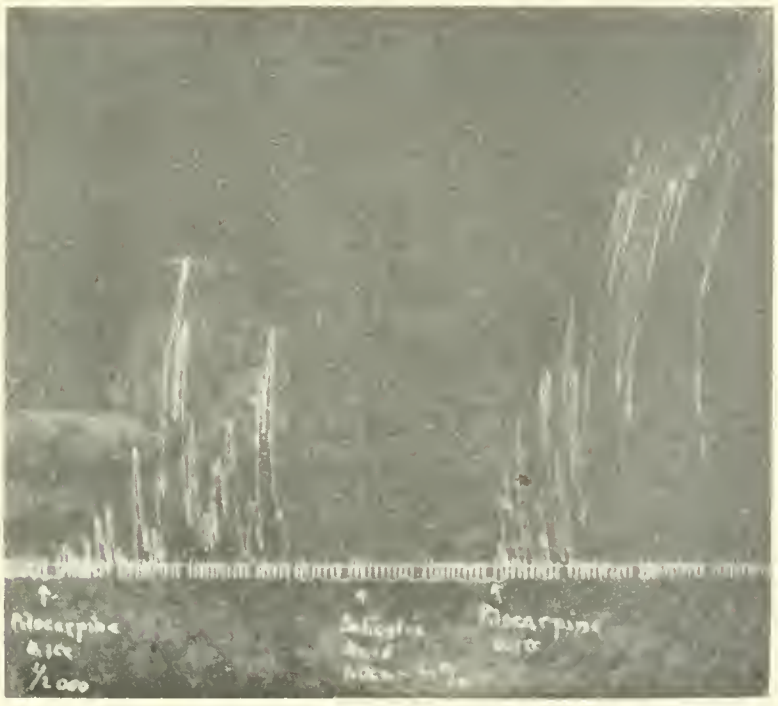


FIG. 3. SENSITIZATION OF RABBIT'S INTESTINAL MUSCLE TO PILOCARPINE (1:50,000) BY SALICYLIC ACID (1:25,000)

taining 0.5 gram cinchophen and 1.5 grams hexamethyleneamine in 5 cc.) to cause an immediate, fleeting and moderate stimulation followed by depression and paralysis of these organs. Using the same mixture containing cinchophen, Ullmann (283) claims the typical effect on excised segments of guinea-pig intestine is parasympathetic stimulation characterized by increased tonus and peristalsis, and antagonism by atropine. These results are supposed to have a relationship to the leukopenia after cinchophen which is assumed to be a "vagus shock."

In concentrations of from 1:800 to 1:160, sodium salicylate was found by Alvarez (322) to cause initial stimulation followed sometimes by depression of excised strips of different portions of rabbit intestine. There was also a tendency to reverse the gradient, which seemed to agree with the tendency of the drug to upset the stomach.

Sensitization of excised intestine by salicylic acid. An interesting sensitization of rabbit intestine by salicylic acid to pilocarpine and barium has been demonstrated by Thienes (323). No effects resulted from the direct application of concentrations of from 1:50,000 to 1:25,000 of salicylic acid, but on the addition of salicylic acid either during or preceding the actions of pilocarpine and barium, the responses to the latter drugs were increased 50 per cent. Figure 3 illustrates a sensitization to pilocarpine. Sodium salicylate, in concentrations of from 1:30,000 to 1:10,000, caused no alterations in the drug responses. The increased response of the intestine to pilocarpine and barium caused by the salicylic acid was probably a cell surface effect in virtue of the lipoid solubility of the salicylic acid, the sodium salicylate giving negative results because it is not lipoid soluble. This sensitizing action is not peculiar to salicylic acid, but occurs with a variety of agents capable of changing the environment of cells and their surfaces, and therefore their functional state and response to stimuli. The result is of interest in connection with the fundamental aspects of allergic phenomena, but should not be transferred directly to explain the peculiar idiosyncrasies toward acetylsalicylic acid occurring in man.

Kidney

The kidney is affected by both salicyl and cinchophen in therapeutic and toxic doses. This is indicated by the increased permeability to uric acid and possibly other metabolites discussed in the section on metabolism, by the changes in urine output, morphology and functional efficiency and by albuminuria.

Diuresis. According to the reports of Blanchier and Bochefontaine (324), Carrieu (325), Blanchier (97), Marrot (203), Huber (326), Chopin (327), Siegert (206), Bardier and Frenkel (328), and Schreuder (262) diuresis is moderately increased in human subjects and animals receiving small and moderate doses of sodium salicylate. Huber's

results on human individuals agreed with those on rabbits with ureteral catheterization. During periods of increased diuresis after 0.03 to 0.06 gram per kilo in dogs, Bardier and Frenkel observed an increase in kidney volume due to vasodilation, and this was followed by vasoconstriction with diminution in flow of urine. Moreigne (260) reported a diminution in diuresis. Wiley (168) observed practically no change in the volume of urine during 24-hour periods after administration of 0.2 to 2 grams per day (average 1 gram) for 30 days. In the after-periods there was a decrease. Cornet (154) found salol to have no influence on diuresis.

On the other hand, full therapeutic doses of sodium salicylate diminish the diuresis in rheumatic and non-rheumatic individuals. This was definitely demonstrated in 13 non-rheumatic and convalescent and five rheumatic individuals by Scott and Hanzlik (329) and Hanzlik, Scott and Reycraft (163). All the subjects were on a constant water intake and the urine was collected in 10-hour periods. The total dosage of salicylate ranged from 9.4 to 22.4 grams administered in 1 gram doses hourly until symptoms of salicylism supervened. The diminution in urine output varied considerably. Some of the subjects showed a temporary increase in diuresis at the time of symptoms of salicylism, but the predominant effect in the majority was a diminution in diuresis. The latter was due in part to diaphoresis, and in part to diminution in renal functional efficiency. The sweating was especially pronounced in rheumatic individuals. The diminution in renal function was demonstrated in the non-rheumatic individuals whose body weight increased unless modified by diaphoresis. The greatest diminution in diuresis was demonstrable at the end of about 30 hours after administration of the salicylate and lasted till the end of about 60 to 70 hours, i.e., practically till the completion of the salicyl excretion. The diminution in renal functional efficiency was indicated by an increase in urea of the blood, a decrease in excretion of phenolsulphonephthalein, and by albuminuria occurring simultaneously with the decrease in urine output. A summary of the results on diuresis and related phenomena after administration of full therapeutic doses of sodium salicylate to human subjects is presented in figure 4.

In a preliminary report, Georgiewsky (330) stated that daily doses

of 0.5 gram of cinchophen for ten days increased the diuresis in human subjects. In normal animals, Starkenstein (331) observed that cinchophen did not increase the diuresis, but it increased the diuresis of epinephrine glycosuria. It merely accentuated an established diuresis.

Albuminuria. The older clinicians generally reported the presence of albumin in urine after the administration of salicyl, but because of the confusion in the methods of testing for albumin, and the origin of the albuminuria, that is, whether febrile or renal, the question remained unsettled. The results of recent studies leave no doubt

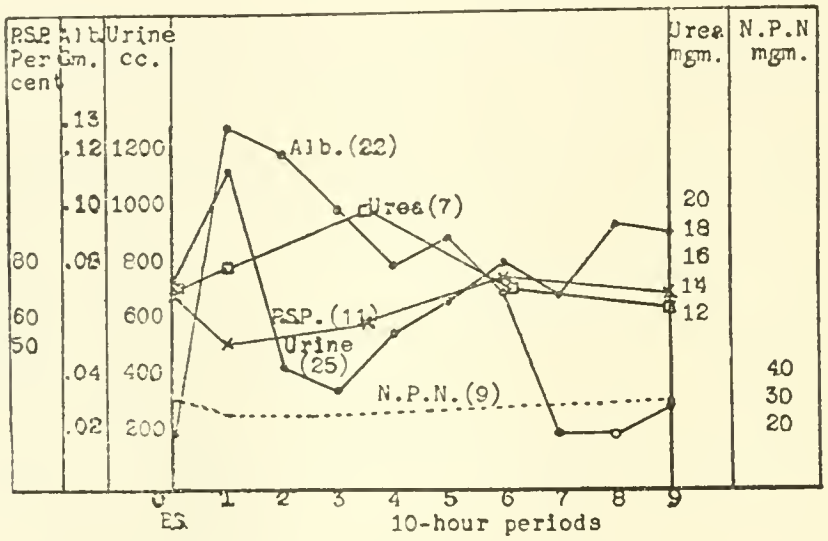


Fig. 4. Diminished Renal Functional Efficiency and Diuresis, and Marked Albuminuria Produced by Full Therapeutic Doses of Sodium Salicylate in Human Subjects

P.S.P., means phenolsulphonephthalein, *Alb.*, albumin in urine, *Urea-N.*, urea nitrogen and *N.P.N.*, non-protein nitrogen, milligrams in 100 cc. of blood, *B.S.*, before salicyl and *A. T.*, at toxicity (symptoms of salicylism). The figures in parenthesis denote number of individuals.

that both salicyl and cinchophen cause albuminuria, and that it is of renal origin at least as far as salicyl is concerned. In adults, it appears that albuminuria is not demonstrable until about 3 grams of these drugs have been taken.

Among the first to report albuminuria after salicyl was Owen (332), who observed a temporary albuminuria in 78 per cent of his cases, and mere traces in 52 per cent, after salicylic acid and sodium salicylate. After therapeutic doses of sodium salicylate, v. Ackeren (333) observed

albumin, blood and casts in the urine of patients, and produced hemorrhagic nephritis in rabbits by the administration of salicylic acid. From an examination of the urines of 33 patients with different clinical conditions, Lüthje (334) found that the majority of the patients (febrile) showed the presence of albumin, hyaline, granular and epithelial casts, leukocytes and cylindroids. The urines were also examined before salicylate administration, and the dose given was usually 5 grams. A return to previous or normal condition occurred in from two to three weeks after the stoppage of the drug. Lüthje ascribed these findings to a nephritis. Practically the same was observed by Kleinberger and Oxenius (335) in a large series of cases. The dose of the sodium salicylate ranged from 3 to 7 grams per day. Knecht, (336) however, differed with Lüthje as to the renal origin of the albumin. He also found traces of albumin and occasional cylinders, but irregularly, and after doses of 0.3 gram in three or more doses these urinary changes did not occur. Brugsch (337) reported that when sodium salicylate was given in divided doses, i.e., 0.5 gram per dose until 5 grams were given per day, no renal irritation occurred. On the other hand, single doses of 5 grams each led to renal irritation in about 5 per cent of the cases. In Wiley's subjects (168) there was a general tendency to increased presence of casts and white blood-corpuscles; albumin excretion was unaffected. The data were few and incomplete. Examinations of urines from more than 100 patients, and from experiments on dogs and rabbits, led Ehrmann (158) to conclude that in only a few instances did albumin occur. The same was true in acute and chronic nephritis, but once an individual showed the presence of albumin in urine, this could be produced again by salicyl. After doses of 0.05 to 0.2 gram per kilo of acetylsalicylic acid, Chistoni and Lapresa (141) noted the presence of small quantities of albumin, casts and white corpuscles in the urine. Mamlock (338) denied the presence of albumin in different clinical conditions. In a statistical study by Hanzlik (298) of clinical cases with different disease conditions, and which received "toxic doses" of sodium salicylate, albuminuria appeared to be more common in the febrile than in afebrile conditions. Hence, it was thought to be of febrile origin.

However, this deduction was not sustained in later studies on normal and diseased individuals by Scott and Hanzlik (329), Hanzlik,

Scott and Thoburn (339) and Hanzlik, Scott and Reycraft (163). These studies were carried out as quantitatively as possible. Full therapeutic doses of sodium salicylate were administered, and a constant water intake was maintained throughout. The urine was examined qualitatively by the heat and acetic acid and ferrocyanide tests, and quantitatively by the gravimetric method of Folin and Denis (340) for the presence of albumin, and microscopically for leukocytes, casts, etc., before and after administration of the drug. The results are illustrated in figure 4 and may be briefly summarized as follows: The administration of sodium salicylate in full therapeutic doses invariably caused the appearance of albumin, white blood-corpuscles and granular casts or cast-like bodies in the urines of normal, rheumatic, convalescent, febrile and afebrile individuals. The albuminuria, therefore, was not of febrile origin. It was due directly to the drug, and a pre-existing albuminuria was aggravated. Quantitatively, the albuminuria reached its maximum at the time of symptoms of salicylism; then it gradually diminished and eventually disappeared, a trace of albumin persisting in those individuals who showed a trace of albumin before the administration of salicyl. These results agreed with those of Hanzlik and Karsner (341) on animals receiving corresponding doses of salicylate. Simultaneously, there were histological changes in the kidneys of the animals indicating nephritis, and a diminution in renal functional efficiency in both the animals and human subjects of these studies leaving no doubt that salicyl albuminuria is of renal origin. Rocco (161) found that 0.1 to 0.3 gram of salicyl salicylate per kilo of animal (dog and rabbit) caused the appearance of renal cells, leukocytes and albumin in urine.

As far as cinchophen is concerned, Hanzlik, Scott, Weidenthal and Fetterman (291) found this drug in full therapeutic doses to cause albuminuria and sometimes the appearance of casts and white blood corpuscles in the urine of the majority of seven individuals that were observed. The total dosage of cinchophen ranged from 4 to 19 grams. Doses of from 5 to 26 grams of neocinchophen gave variable results though albuminuria occurred in about one-half of the nine individuals that were observed. Albuminuria was claimed to be generally absent after small and large doses (up to 16 grams) of neocinchophen in a series of over 19 rheumatic and other patients reported by Barbour, Lozinsky and Clements (292).

Bicarbonate on salicyl albuminuria. Glaesgen (342) claimed that, in human subjects, the administration of from 6 to 10 gram daily doses of sodium bicarbonate caused albuminuria to disappear after 3 to 6 gram doses of acetylsalicylic acid and the therapeutic effects were not lessened. Frey (343) claimed that large doses of salicylate in rabbits and dogs led to albuminuria, when the urine was rendered acid (by administering hydrochloric acid), and that albumin was absent in urine rendered alkaline by the administration of sodium acetate. On the other hand, Ehrmann (158) claimed that alkalinity had no influence on the excretion of albumin when albuminuria occurred, but he was not convinced that salicyl always caused albuminuria. The question was definitely settled in the negative by the observations of Hanzlik, Scott and Thoburn (339) and Hanzlik, Scott and Reycraft (163) on 7 human subjects receiving full therapeutic doses of sodium salicylate together with equal doses of sodium bicarbonate up to a total of 16 grams. There was no demonstrable influence on the albuminuria and the diminution in renal functional efficiency produced by the salicyl despite the high degree of urinary alkalinity persisting throughout.

Renal functional efficiency. There is no doubt that salicyl and cinchophen increase the permeability of the kidney to uric acid, and possibly to urea, creatinine and chloride, though there is considerable uncertainty about the latter constituents of the blood. It appears that the uric acid effect is not obtained with doses of less than 3 grams of sodium salicylate. With medium and full therapeutic doses, an occasional individual responds with an increase in excretion of water (urine output). The cause of this increase is not known; it may be only a salt effect. However, in the majority of human individuals and in animals receiving full therapeutic doses of salicyl, the net result is a diminution in renal functional efficiency as indicated by the considerable reduction in diuresis discussed above, by a definite diminution in the excretion of phenolsulphonephthalein and by an increase in the urea-nitrogen of the blood. This was shown in the studies of Hanzlik, Scott and Thoburn (339) and Hanzlik, Scott and Reycraft (163) on human subjects and of Hanzlik and Karsner (341) on animals. A summary of the results on human subjects is presented in the form of curves in figure 4, and shows quite plainly that during the decrease

in excretion of urine and phenolsulphonephthalein there was simultaneously an accumulation of urea in the blood. The non-protein nitrogen of the blood of the nine subjects studied was diminished somewhat during the increase in urea. It may be that the increase in the escape of the uric acid fraction of this nitrogenous portion was responsible for the change. However, in animals, the non-protein nitrogen and urea of the blood were both definitely increased. The uric acid was not estimated in any of these experiments. The changes in blood urea and in the excretion of phenolsulphonephthalein and water were demonstrable at the time symptoms of salicylism appeared, reached their maximum at the end of about 29 to 30 hours, and returned to their previous level at the end of about 60 to 70 hours after administration of the salicylate. Albuminuria was present simultaneously with all these changes in both the animals and human subjects. In the animals there were, in addition, definite indications of nephritis histologically, leaving, therefore, no doubt of renal injury from the salicyl.

Cinchophen, in full therapeutic doses, was found by Hanzlik, Scott, Weidenthal and Fetterman (291) to cause a definite diminution in the excretion of phenolsulphonephthalein together with albuminuria and other changes in the five human subjects that were studied. Neocinchophen, in full therapeutic doses, was more variable, causing a diminution in the excretion of phenolsulphonephthalein in about one-half of the 9 subjects that were observed, and who showed albuminuria at the same time. On the whole, the injury to renal function was somewhat less after cinchophen and still less pronounced after neocinchophen than after sodium salicylate in corresponding doses. In 8 patients receiving ordinary doses of cinchophen, Eisner (344) found the excretion of sodium chloride and total nitrogen decreased, both constituents returning to normal on withdrawal of the drug. In several cases there was later a compensatory increase in these urinary constituents. The uric acid in urine was always increased. An analogy between the action of cinchophen and calcium on renal function could not be established.

Nephritis. This has been demonstrated with salicyl in both animals and man. No studies with cinchophen have been reported. The occurrence of albuminuria, casts and sometimes hematuria, and the diminution in renal functional efficiency discussed above, suggest a

definite nephritis after salicyl. However, few human subjects receiving salicyl in therapeutic doses come to autopsy and, therefore, histological studies of human kidneys are practically non-existent. About the only reports on the human kidney are those of Vinci (345) and Wetzel and Nourse (273); the remaining three reports deal with animal kidneys.

Vinci (345) observed a toxic parenchymatous nephritis at necropsy of a man 45 years of age, who died 31 hours after the ingestion of 35 grams of sodium salicylate. There was marked congestion of all the renal vessels, particularly in the glomerular tuft, albuminous precipitate in the subscapular space, interstitial hemorrhage, cloudy swelling and extensive necrosis of the tubular epithelium, most marked in the cortex; but as the necropsy was performed 2 days after death, and as the kidneys sent to Vinci were imperfectly preserved in alcohol, little importance can be attached to the histological changes. Experimenting with dogs, guinea pigs and rabbits, he found that massive single doses, and frequently repeated large doses, led essentially to the same changes as he described in the human kidney. For the most part the drug was given by mouth, but in a few instances, intravenously and subcutaneously. He states that the lesions were most marked in dogs, less in guinea pigs, and least in rabbits. Lüthje (334) gave repeated doses of sodium salicylate to 2 dogs, and both showed cylindruria, one showing albuminuria. The kidneys showed general congestion, interstitial hemorrhage, cloudy swelling, fatty degeneration, cast formation and, in the subscapular space of the glomerulus, a homogeneous coagulum. Nephritis was ascribed to salicylate by Quenstedt (346) though on insufficient grounds. Hanzlik and Karsner (341) made observations on the morphology and functional efficiency of the kidneys of dogs, cats and a rabbit receiving doses of sodium salicylate corresponding to the full therapeutic, or so-called "toxic," dosage used in human individuals, i.e., about 0.23 gram per kilo. In some cases only one dose was administered and in other cases repeated doses, the salicylate being usually injected hypodermically in 10 per cent concentration. In a few instances the drug was also given by mouth. The renal changes were the same as when the drug was given hypodermically. As a rule the animals were observed for 2 to 3 days before the experiment was begun, since they

almost invariably showed some evidences of albuminuria. Studies of changes in the albuminuria and fixed elements in urine and in the urea and non-protein nitrogen of the blood were made at the same time. Then the animals were killed, necropsy performed and kidneys at once placed into a fixing solution. Besides the albuminuria and diminution in renal functional efficiency which occurred in all of the

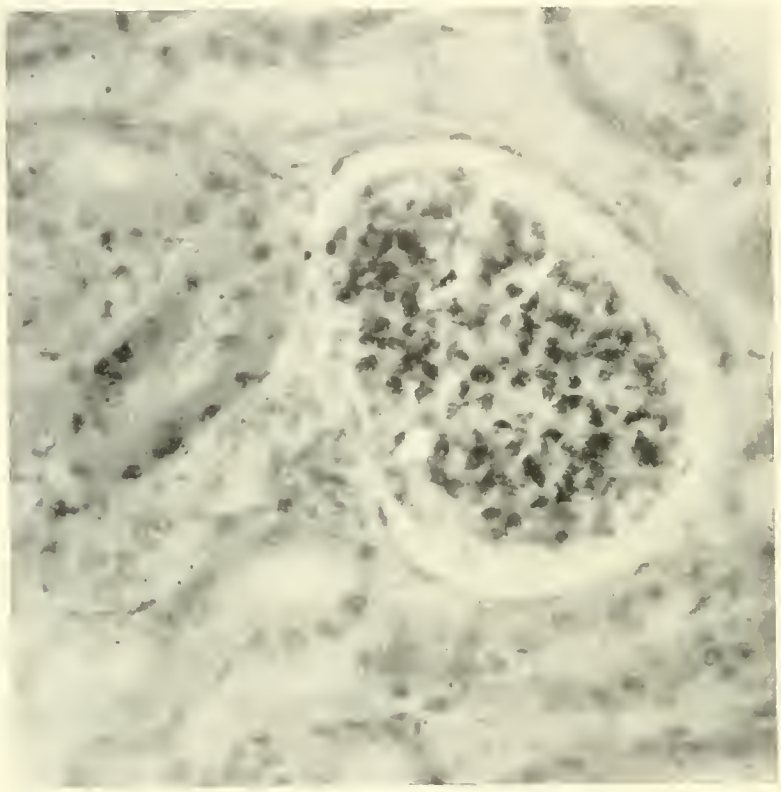


Fig. 5. Section of Dog Kidney Illustrating Tubular Nephritis Following Hypodermic Injection of Sodium Salicylate (0.3 Gram per Kilo)

Shows slight involvement of the glomerular tuft, which, although not enlarged, is richly cellular and devoid of blood. The tubular epithelium shows cloudy swelling and the lumina contain granular albuminous precipitate.

eleven animals studied and were described above, there were definite morphological changes in the kidney. The renal lesion varied in severity from simple cloudy swelling of the epithelium of the proximal convoluted tubule to extensive cloudy swelling of all the cortical parts of the tubules, associated with an acute intracapillary glomerulitis, the latter process being denominated as an acute tubular nephritis. These changes agree essentially with those of Vinci, but, contrary to

his claim, there were no material differences in the susceptibility of dogs and cats. Vinci's failure to find the more marked glomerular lesion was probably due to the fact that most of his animals died or were killed after only 7 or 8 hours. Figure 5 illustrates the changes in a dog kidney from the experiments of Hanzlik and Karsner. The recent report of Tocco-Tocco (347) on renal changes from salicyl is in essential agreement with the results just given, the most serious alterations being in the glomeruli, tubules and vasi. On direct treatment with ferric alum these regions gave the most marked color reactions for salicyl, from which Tocco-Tocco concludes that the salicyl accumulates in the regions most markedly affected morphologically.

In their case of fatal poisoning in an infant 21 months old who drank about 10 cc. of oil of wintergreen (methyl salicylate), Wetzel and Nourse (273) found cloudy swelling and fatty degeneration in the tubular portion of the renal cortex, but the glomeruli were unaffected. The kidney parenchyma showed a generalized hyperemia and hemorrhages. The urine showed a faint trace of albumin and occasional hyaline casts.

The only report on cinchophen is that of Nicolaier and Dohrn (28), who state that a rooster which had received 44 grams of the drug in 66 divided doses showed no histological changes in any of the organs, but no details are given.

Salicyl edema

Systemic. This was described by Hanzlik, Scott and Reycraft (163), who observed it in 9 human subjects after administration of full therapeutic doses of sodium salicylate. It is characterized by an increase in body weight demonstrable about 20 hours after the beginning of administration of the salicylate and persists until the salicyl excretion is completed, that is, till the end of about 80 hours, or $3\frac{1}{3}$ days. Simultaneously there occur a marked diminution in diuresis, a diminution in phenolsulphonephthalein excretion, an accumulation of urea in the blood and albuminuria, all of these elements returning to their previous levels with the disappearance of the edema. Since the diminution in renal functional efficiency generally makes its appearance before an actual increase in body weight is demonstrable, and later coincides with it, it seems that the renal factor plays an

important rôle in the production of the edema. The retention of water occurs mainly in the tissues, since no dilution of the blood is demonstrable by hemoglobin estimations. Diaphoresis may modify the degree of the edema, but it is not modified by the administration of bicarbonate together with salicylate in large doses (up to 16 grams) and sufficient to maintain the urine alkaline. Swift (348) states that he has not observed as marked changes with respect to edema and renal injury as have just been described, but does not deny their

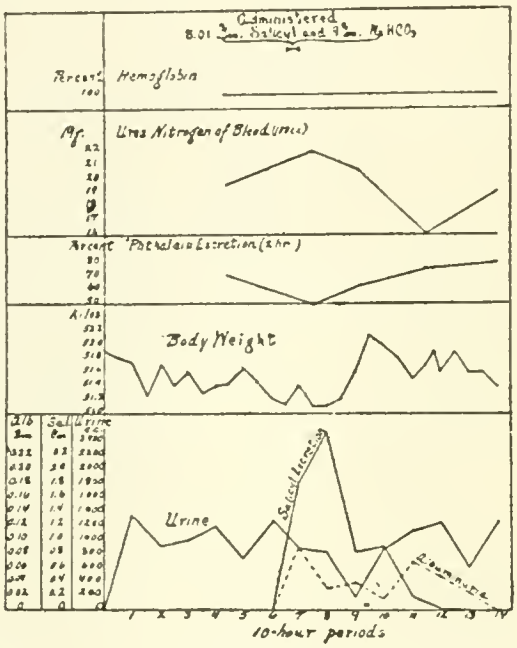


FIG. 6. SYSTEMIC EDEMA AFTER A FULL THERAPEUTIC DOSE OF SODIUM SALICYLATE IN A HUMAN SUBJECT (PATIENT 28)

Shows increase in body weight, accumulation of blood urea, diminished excretion of phenolsulphonephthalein, diminished output of urine, albuminuria, excretion of salicyl and unchanged hemoglobin.

occurrence with large doses of the drug. The typical phenomenon, including the various changes in blood, urine salicyl excretion and body weight, is illustrated in figure 6.

Pulmonary edema. Chatin and Guinard (349) produced pulmonary edema together with increases in salivary, bronchial, nasal and digestive secretions in animals by injecting methyl salicylate (0.025 gram in dogs and 0.047 gram in rabbits) intravenously; the circulation was only moderately affected, and respiration stopped before the heart. Chanoz and Doyon (350) produced the edema in dogs by injecting

amylsalicylic ether intraperitoneally or intravenously (0.5 to 0.8 gram per kilo); death occurred from arrest of respiration and convulsions. According to Teissier and Guinard (351), the cause of the edema was not due to an increase in pulmonary arterial pressure because the pressure was only slightly increased in curarized animals receiving artificial respiration and injections of the ester. That is, mechanical difficulties alone were insufficient, although they facilitated the occurrence of lung edema. Additional important elements were vasodilation and intoxication. These authors injected from 2 to 7 cc. into the jugular veins in 20- to 24-kilo dogs. The vascular changes might be expected to occur from an irritant volatile oil such as methyl salicylate. Apparently the acute pulmonary edema of methyl salicylate differs as to cause from the systemic edema of sodium salicylate, although it is possible that the vessels are also affected in the latter type.

Local edema of ears. The edema action of salicyl esters might be expected to hold for other regions besides the lungs. This was demonstrated by Dreser (357), who applied mesotan (methyl oxymethyl salicylate) directly to rabbit ears. The swelling of the ears was determined by measuring the volume of water displaced on immersion of the organs into cylinders. The application of 1 gram mesotan to an intact ear caused it to increase 14 cc. in volume at the end of 22 hours, and to 18 cc. at the end of 28 hours. It took 4 days before the ear returned to its original size. Mesotan in oil did not cause as much swelling owing apparently to competition of the lipoid solvent with the ear. The ear experiments indicate the basis for the use of salicyl esters locally as irritants. As compared with methyl salicylate, spirosal (monoglycol salicylate) and possibly ethyl salicylate are less irritating. All of these esters are useful as counterirritants.

Circulation

Small therapeutic doses of salicyl and cinchophen do not affect the circulation demonstrably except in susceptible individuals who respond with some acceleration of the pulse and consciousness of the precordia. Full therapeutic doses cause some depression though this is comparatively unimportant, while large and toxic doses definitely depress and finally paralyze the circulation in the manner of the

coal-tar derivatives in general. However, clinical investigations with modern methods are practically non-existent. The following summary contains mainly the results of studies on animals and excised organs.

Blood pressure. After intravenous injection of either salicylic acid (0.023 gram) or its sodium salt (0.08 gram) in rabbits, Köhler (353) observed a fall of blood-pressure after section of the vagi, depressors and cervical cord, and this was proportional to the dose. No such effects were observed when the drug was given by mouth, but in dogs gastric administration was followed by a fall of blood-pressure, with retardation of the pulse. Later Köhler (354) found that intravenous injection of doses from 0.5 to 0.75 gram in dogs resulted in a rise of blood-pressure with retardation of the pulse and respiration, but this was due to asphyxia, since in curarized animals with artificial respiration the effects were those of depression. Moreover, further injections caused the blood pressure to fall progressively and the pulse was slowed. Köhler denied the claim of Danewski (355) that salicyl stimulates the vasomotor center and the vagus, acting like digitalis. Oltramare (356), also having obtained a rise in arterial pressure with moderate doses, concluded it to be of central vasomotor origin. Clinically, Maragliano (357) noted usually an elevation in the pressure; never depression.

According to Chatin and Guinard (349), intravenous injection of methyl salicylate, in absolute doses of 0.025 gram in dogs and 0.047 gram in rabbits, caused a marked but transient fall of blood pressure (carotid) followed by a fleeting rise, followed later by a fall with acceleration of the pulse. The cause of these changes was not determined, but it was stated that other methods of administration did not affect the circulation. The action on pulse was feeble and unimportant with small doses of the ester. Essentially the same effects of methyl salicylate on blood pressure were observed by Teissier and Guinard (351).

Intravenous injections of large doses of cinchophen in cats and dogs cause stimulation of the vagus and vasomotor centers followed by depression with corresponding effects on the blood pressure (230). Using "leukotropin," a mixture of cinchophen and hexamethyleneamine, injected intravenously in a dosage corresponding to about 0.2

gram cinchophen in rabbits, Ullmann (283) observed a fleeting fall of blood pressure with slowing and increase in volume of the heart. This was prevented by large, but not by small, doses of atropine. The fall of blood pressure appeared to be due to cardiac slowing from vagus stimulation. Section of the vagi and decerebration did not prevent the action, and therefore the vagus stimulation was peripheral. This agreed with parasympathetic stimulation of the intestine. Epinephrine antagonized the circulatory action of cinchophen. In man, there were no effects on blood pressure and the heart from the intravenous injection of 10 cc. of the mixture, containing about 1 gram of cinchophen. On the other hand, Starkenstein (320) claims that the lowering of blood pressure caused by intravenous injections of minimal effective doses of cinchophen in cats, dogs and rabbits is not of vagal origin.

Intact heart. As a rule, there is no effect with small, but depression occurs with large, doses of salicyl and cinchophen. In frogs, Istomin (358) and Istomin and Weliky (359) observed at first a slowing of the pulse, later an acceleration after small doses. This only returned to normal in 60 to 100 minutes if the spinal cord was not cut. The heart stopped in incomplete systole and was unaffected by atropine or electrical stimulation. Blanchier (97) observed an increase in rate and strength of the heart beat during a small rise in blood-pressure which was probably of central origin. With increase in dosage, this was followed by arrhythmia, irregularity, diminution in strength of pulse with sudden stoppage of the heart and cessation of respiration. In guinea pigs, Houghton (77) found ethyl salicylate in doses of about 0.0015 gram per gram body weight to slow the heart, large doses producing cardiac standstill. This action was presumably on the cardiac muscle direct. The effects of methyl salicylate (0.0007 gram per gram body weight) were more pronounced on account of its greater toxicity. The results of Borrisow (360) appear to be in accord with those of others.

With sodium cinchophen injected intravenously, Starkenstein (320) observed a temporary slowing with increase in amplitude of the pulse in rabbits, but not in cats and dogs. He denies a vagus type of pulse from this drug. Essentially the same changes in rabbits were obtained by Ullmann (283) with "leukotropin," but he interprets the mechanism

as a "vagus shock," and states that Starkenstein's injections resulted frequently in thrombosis.

Clinically, Sée and Lahalle (361) observed no change in pulse-rate and rhythm with comparatively small doses of sodium salicylate. With small as well as larger therapeutic doses, Maragliano (362) noted an increase in the strength of the heart beat, and the systolic pressure reached its maximum in 2 hours and returned to normal in 3 to 5 hours; the normal dicrotism usually being accentuated. Doses of from 4 to 19 grams of cinchophen were found by Hanzlik, Scott, Weidenthal and Fetterman (291) to slow the pulse rate about 25 per cent in both febrile and afebrile subjects (eight). The slowing, therefore, was not the result of antipyretic action. Neocinchophen also slowed the pulse in 4 febrile subjects.

Perfused heart. The isolated heart is not readily poisoned by salicyl as indicated by the fact that rather high concentrations are necessary for definite effects. Such concentrations are much higher than the concentration of about 0.02 per cent occurring in blood after full therapeutic, or clinical "toxic," doses.

Faval (363) found low concentrations ineffective for the isolated frog heart; high concentrations paralyzed. Friedrichsen (128) noted that concentrations of from 0.04 to 0.05 per cent of sodium salicylate in Locke's solution, and of 0.2 per cent in blood, were necessary to cause stoppage of the perfused frog heart. Lower concentrations reduced the rate and strength of the heart beat. In the living frog, a concentration of 0.4 per cent sodium salicylate in the blood stream was necessary to cause stoppage. In the intact rabbit a concentration of 0.12 per cent of sodium salicylate had no effect, but with higher concentrations the pulse was slowed, blood-pressure fell and the heart stopped. The excised and perfused heart was depressed by a concentration of 0.12 per cent. Death occurred when the concentration of salicylate reached 0.13 per cent in the blood after administration of the drug by mouth, but 0.06 and 0.08 per cent concentrations in the blood were endured for 24 hours without death. Friedrichsen noted also that beef and rabbit serums always contained more salicyl than the erythrocytes, the proportion in serum to whole blood being 1.2 to 1. Salant and Johnson (364) found that perfusion of the isolated frog and turtle hearts with a concentration of 1:2000 sodium salicylate had no

effect or stimulation; 1:1000 at the end of 10 to 15 minutes caused considerable depression, and there was a slight improvement sometimes on discontinuation of the perfusion, but complete recovery was not observed; 1:500 and 1:250 caused still greater depression. Okushima (365) reports that the action of salicylic acid and its sodium salt on the heart resembles that of other aromatic acids and their salts, and suggests that the depressant action of salicylates is due to liberation of the acid by metabolites given off by excised organs, especially as an effect is more rapidly obtained with an organ in activity than when it is at rest. However, this implies a much higher degree of acidity in tissues than probably exists.

In a later report, Okushima (366) stated that the esters of various aromatic acids have a more pronounced effect on the isolated heart than the corresponding sodium salts of their acids, but on hypodermic injection in mice the salts were more effective than the esters. The latter would seem to be a matter of solubility. In the experiments of Salant and Johnson (364), heart block occurred in 2 to 3 minutes after perfusion of the isolated frog heart with concentrations of 0.7 per cent or less of the methyl and ethyl salicylates; the effect was promptly removed with Ringer's solution. Acetylsalicylic acid, in concentrations of 1:4000 to 1:2000, caused marked depression, but when the pH of the solution was maintained at 7.4 or 7.5 stimulation occurred, and, therefore, this derivative was less toxic than the sodium salt of salicylic acid. Mendenhall and Camp (367) observed that acetylsalicylic acid had a stimulant effect on the intact and perfused frog heart as indicated by lowering of the threshold to electrical stimulation, the action of the drug being direct and not due to paralysis or inhibition of the vagi. The dosage in frogs was 0.25 mgm. per gram body weight. Injection of doses comparable to the therapeutic in cats accelerated the heart and increased the blood pressure independently of the vagi. They concluded that the drug is depressant to the heart only in concentrations much higher than would be likely to occur even after large doses. Using Williams' perfusion apparatus and defibrinated ox blood, Dreser (11) estimated the cardiac output and work of the frog's heart during perfusion with 0.2 per cent sodium salicylate. This salt left the force unchanged, but reduced the pulse volume as compared with a control solution, while 0.25 per cent

acetylsalicylate increased the work through an increase in pulse volume though the force remained unchanged. These results were essentially similar to those of Salant and Johnson.

Ullmann (283) observed that perfusion of the isolated frog heart with "leukotropin" resulted in slowing of the rate and diminished systole; in other words, direct depression. The effects were antagonized by calcium. Rotter (255) claims paralysis of cardiac nerves in the frog's heart treated directly with cinchophen, the heart usually stopping in systole, but this seems entirely out of line with the results of Ullmann and of Starkenstein discussed above. Hydroatophan, according to Pohl (368), does not possess the action of cinchophen on the amphibian heart, but causes intense spinal and peripheral stimulation resulting in tetanus and fibrillary contractions.

The main criticism of the majority of reports on the perfused heart is the failure to take into consideration the changes in the perfusion fluid resulting from the addition of cinchophen and salicyl and their derivatives. These changes consist essentially of the alterations in reaction (pH) from cinchophen and the salicylic and acetylsalicylic acids, which would reduce the alkalinity, and from the sodium salts of the compounds, which would increase it, since they are salts of relatively weak acids. In the case of "leukotropin," the presence of hexamethyleneamine might alter the action of cinchophen. In other words, the cardiac effects observed may have been due to pH changes, etc., rather than to the drugs themselves.

Blood vessels. These tend to be relaxed by all concentrations. Dreser (11) found the vessels of frogs with cord destroyed relaxed on perfusion with sodium salicylate and sodium acetylsalicylate; the concentrations were not stated. Dilatation of the peripheral vessels from sodium salicylate in higher animals was observed by Wiechowski (369). The intracranial vessels were constricted, but the homologues, benzoic and p-oxybenzoic acids, and acetylsalicylic acid and methyl salicylate had no such action. In his direct observations of capillaries in the frog's mesentery, Ikeda (280) saw no depression of the circulation here from the injection of cinchophen, while quinine depressed. A relaxation of vessels from salicyl and cinchophen might be expected as was found to be the case with a variety of aromatic substances by Kondo (370). The action is on the smooth muscle itself and results in

paralysis with concentrations which oppose and prevent the actions of barium and epinephrine. In experiments with excised vessels themselves or those of excised organs, it is necessary to keep in mind the changes in pH of the fluid just as in the case of the isolated heart. Unfortunately, this was not the case in the few reports discussed. Nevertheless, the results generally agree with those of injection experiments which are not subject to the same local changes.

Respiration

Ordinary therapeutic doses of salicyl and cinchophen do not appreciably affect the respiration, but full therapeutic doses tend to accelerate and deepen it. Toxic doses depress and finally paralyze. The early effects appear to be of circulatory origin, since they occur at a time when the pulse is slowed and the blood-pressure tends to fall. Dosage is an important factor, but clear cut analyses are still lacking, and the available reports are somewhat contradictory.

After gastric administration of sodium salicylate and salicylic acid in dogs, a slowing of the respiration was noted by Köhler (353, 354). In guinea pigs and dogs, Blanchier (97) noted an acceleration at first, followed by interrupted respiration, dyspnea and death from asphyxia. Practically the same was observed by Chirone and Petrucci (195). Danewski (355) claimed that, if the vagi were cut before injection of the salicylate, the respiration was only slightly accelerated. This then returned to the same rate as in the control untreated animals with sectioned vagi, the drug, therefore, acting independently of the vagus mechanism. The marked increase in respiration and air hunger after hypodermic and intravenous injection of sodium salicylate in animals were found by Hurtley and Trevan (274) to be similar to those of acetoacetate and ethyl acetone, enol compounds, which reproduce the symptoms of diabetic acidosis. Small doses of ethyl salicylate in guinea pigs at first slowed then accelerated the respiration, according to Houghton (77); large doses paralyzed. Houghton also observed that no absorption of this ester took place by the respiratory passages. In dogs and rabbits, Chatin and Guinard (349) observed constant effects after intravenous injection of methyl salicylate, namely, acceleration of respiration and dyspnea, which increased with the degree of poisoning, and finally the respiration stopped before the heart. In

these experiments the circulatory changes were relatively unimportant.

Clinically, Deseille (287) reported no change in respiration after therapeutic doses of salicylate. Ullmann (283) states that cinchophen, in the form of "leukotropin," increases the asthmatic attacks in man.

Skeletal muscle and nerve

The sodium salts of salicylic acid and cinchophen have no demonstrable direct effects on skeletal muscle, but the free acids depress. The convulsant action after administration is analogous to that of phenol and entirely central in origin. In the intact frog, Livon (371) reported that sodium salicylate caused first an increased and later a decreased irritability. Acceleration of rigor mortis was reported by Blanchier in 1879 (97), and this is characteristic of convulsants in general. Recently, Schüller (322) demonstrated the absence of toxic action from the sodium salts of salicylic acid and other aromatic acids on excised frog muscle. When carbon dioxide was passed through the solutions of the sodium salts, the toxicity increased with the increase in lipoid solubility of the acid liberated, and, therefore, in accordance with physical-chemical behavior. The ultimate effect, however, was determined by the chemical constitution of the acid. These results agree, in general, with those of Thienes (323) on smooth muscle, who found that the alterations in functional response were produced only by the lipoid soluble salicylic acid and not by the sodium salt. Schüller (372) finds, also, that sodium salicylate, sodium cinchophen, and sodium cumarate can remove the actions of caffeine and veratrine (contracture) on skeletal muscle. He thinks the antagonism to these alkaloids rests on the same basis, and claims it is rather uniform for all agents with the hydroxyl group in the ortho position, including the antagonists tried. The presence of calcium hinders the antagonism. A definite explanation of the antagonism is not at hand, but in the case of the caffeine-salicylate antagonism, it seems the caffeine is removed through the formation of the complex, though very soluble, caffeine sodium salicylate. The same is suggested as the explanation of the veratrine-salicylate, and possibly veratrine-cinchophen, antagonism.

Salicyl and cinchophen do not act on peripheral nerve structures.

They do not produce local anesthesia. The alleviation of pain locally, claimed by some for methyl salicylate, especially on joints, is probably due to a counter-irritant action, although it is true that Dreser (352) demonstrated some depression of reflexes from frog's skin treated with the undiluted ester. There was some prolongation of the reflex time elicited by electrical stimulation of the skin. A saturated solution of the ester in water, however, was ineffective. The analgesic action of these drugs is mainly central, and analogous to that of other coal-tar derivatives such as acetanilide and antipyrine. The depression is marked enough to relieve the pains of neuralgia and arthritis and headache, but not enough even with full therapeutic doses to permit minor operations. A mild hypnotic action is experienced by some individuals, especially with acetylsalicylic acid, but this does not compare with the activity of such hypnotics as morphine, bromide, barbital, etc. Saturated vapors of methyl salicylate were found by Dreser (362) to completely narcotize mice; depression was still present at the end of 20 hours, complete recovery occurred on the third day. Fish were narcotized in 5 minutes by a concentration of 0.003 per cent in water, but not by an equal concentration of mesotan, the latter being effective, however, in 2 minutes when twice the concentration was used.

Uterus

Emmenagogue action. After large doses of salicylates, uterine hemorrhages, miscarriages and premature births have occurred. These have usually occurred during the treatment of rheumatic fever and it is probable that the febrile condition was responsible in part at least. Strong menstrual and abortive effects due to salicylates have been reported by Mattos (373). According to Pullmann (374), Schuchardt (375), Wacker (376) and Linhart (377) hemorrhages from the mucosae occur in some individuals after taking salicyl. It has been suggested by Binz (378) that this may be the basis of frequent or excessive menstruation, and of miscarriage or premature birth, after salicyl.

Excised and intact rabbit uterus. The occurrence of abortion seemed to be supported by Binz's experiments (378) on pregnant rabbits. Five out of 15 rabbits aborted after doses of salicyl which had no other action. Binz left the mechanism unexplained, but advised

caution in the use of the drug in cases with a tendency to miscarriage or hemorrhage. Gunn and Goldberg (379) observed the effects of sodium salicylate on the motility of excised and intact pregnant and non-pregnant uteri of rats, guinea pigs, cats, and rabbits. They demonstrated a definite stimulating action. The action was not powerful; on the isolated uterus it was only half as strong as sodium carbonate and about one-fortieth as strong as quinine. A concentration of 1:1000 of sodium salicylate added directly to the excised strips always stimulated as indicated by an increase in tonus, or rate, or induction of movements in a previously quiescent uterus. Strengths of 1:2000 and less had some stimulating action, while 1:500 caused a primary stimulation followed by depression. The effects were reversible and probably muscular. Since the concentration of sodium salicylate in blood after full therapeutic doses is about 0.024 per cent, and at least 0.05 per cent was necessary in Gunn and Goldberg's experiments to cause slight stimulation, it does not seem that the muscular action alone is responsible for abortions occurring in rheumatic fever. Gunn and Goldberg noted some fleeting stimulation of the intact uterus after doses up to 0.2 gram per kilo in nearly two-thirds of their 21 experiments. The effects consisted of an increase in rate or amplitude of contractions. In over a third of the experiments salicylate had no effect on the uterus. When the drug was given in divided doses, as it is in rheumatism, the stimulation was not obtained. From their results, Gunn and Goldberg concluded against the importance of salicyl as an abortifacient unless the uterus is specially sensitive, and suggested that abortion occurring in rheumatic fever is the result of the fever, or it may arise in some cases from hemorrhage into the uterus. This seems correct, but it is still possible that the human uterus is more sensitive than the animal uterus to both the permeability and muscular actions of salicyl, or that abortion is caused indirectly through effects of salicyl on the fetus, for instance, by inducing respiration and causing struggling and death. Therefore, Binz's advice is still valuable.

Auditory organs

Tinnitus aurium is one of the symptoms of salicylism, or cinchonism, occurring after full therapeutic doses of salicyl and cinchophen in

human subjects. In rabbits, mice and guinea-pigs poisoned with sodium salicylate, disturbances in the auditory apparatus do not occur from hemorrhages in the labyrinth as thought by Kirchner (380), but rather as according to Blau (381) and Haike (382), who also made experimental studies and found that there is injury to ganglion cells in the spiral ganglion, and particularly in the vestibular ganglion, similar to that produced by quinine as observed by Wittmaak (383). This injury consists of an alteration in the staining properties of the nerve cells; alteration in the distribution of the Nissl bodies, partial disappearance and sometimes destruction of the entire nerve cell. These changes are not characteristic of salicylates. They can also be produced by asphyxia, strangulation, convulsions and such drugs as arsenic and strychnine. Lindt (384) observed no degenerative changes in the auditory nerve after administration of salicylate and quinine, and states that it is as yet unknown where these drugs act to produce the ear symptoms. Congestion may be responsible for them, for dilatation of cerebral vessels after salicyl and related drugs has been reported by Berezin (385) and Wiechowski (369).

Tinnitus aurium and other symptoms of salicylism occurred in only 20 per cent of the subjects receiving neocinchophen as compared with the majority or 65 per cent of those receiving cinchophen in the series of Hanzlik, Scott, Weidenthal and Fetterman (291). This did not seem to be a matter of dosage. Neocinchophen, therefore, probably has some advantage in this respect over the salicylates and cinchophen. No experimental studies of the ear have been made with these drugs.

Sweating

Sweating occurs after small and full therapeutic doses of salicyl and cinchophen. This is sometimes quite marked with the relatively small doses of acetylsalicylic acid used in the treatment of colds. In febrile conditions it is usually more marked than in the afebrile, but the sweating may be so marked after full therapeutic doses in the afebrile as to reduce the body weight. This was observed by Hanzlik, Scott and Reycraft (163). Justi (386) observed that a profuse sweat takes place in fifteen minutes after administration of salicyl in fever, and Goltdammer (387) and Ewald (76) noted that the perspiration may be so marked as to cause exhaustion. The doses of salicyl em-

ployed by Hare (cit. H. C. Wood (388)) in a clinical study of this question did not produce sweating. The diaphoretic action may be connected with the peripheral vasodilation caused by these drugs, and, in fever, the fever has probably an added influence. The sweating is of course important in the antipyretic action of the drugs.

Temperature and heat regulation

Salicyl and cinchophen are prompt and efficient antipyretics. In 1874, Kolbe (29) first suggested an antipyretic action for salicyl on the basis of certain other pharmacological resemblances to quinine, which however had nothing to do with a temperature action, and Buss (389) demonstrated its antipyretic action in 1875. Formerly the antipyretic action was attributed to depression of the heart and respiration, but this was incorrect because the temperature action occurs before cardiac depression is demonstrable, the latter requiring much higher concentrations than occur in the body after therapeutic doses. Salicyl undoubtedly dilates the peripheral vessels including those of the skin, probably through central depression, and this together with the accompanying sweating, at least in fever, suggests the probable mechanism of the antipyresis. It was seen that protein metabolism is somewhat increased, if anything. Depression of metabolism (heat production), therefore, is excluded as indeed is indicated by the results of various studies of this feature. The mechanism, therefore, appears to be similar to that of acetanilide and antipyrine, and consists essentially of increased heat dissipation. Whether it resembles or differs from quinine it is difficult to say at present because the long-accepted depressant action of quinine on metabolism has been seriously questioned by Hardikar (390). It seems to be generally agreed that quinine is a more powerful antipyretic than salicyl and cinchophen. Sweating is not indispensable to the antipyretic action, since perceptible sweating does not always occur during the antifebrile action, and again marked sweating occurs after salicyl in normal individuals without as a rule a lowering of body temperature. Although the following summary shows that as yet there is not a complete agreement on the effects of salicyl on normal temperature, the tendency of the results is toward the negative side, animals being more variable than man as might be expected.

Normal animals. Practically negative results with salicyl on body temperature in normal animals have been reported by the following investigators; Buss (389), Fürbringer (53), Barbour and Herrmann (391) in dogs, Morinaka (392) in dogs and rabbits, and Dreser (11) in rabbits. The reason for the negative effects in the experiments of Barbour and Herrmann was the absence of blood dilution, which, however, occurred in febrile dogs. The blood dilution appeared to be dependent on the increase in concentration of blood sugar. The average maximum increases in sugar after acetylsalicylic acid and sodium salicylate were 25 and 48 per cent, respectively, in the normal, and from 44 and 50 per cent, respectively, in the febrile, dogs. The dosage of sodium salicylate was 0.25 gram, and of acetylsalicylic acid 0.5 gram, per kilo. Slight temperature increases after both drugs were observed in some of the dogs, this preventing the blood dilution, if anything, and therefore favoring the theoretical explanation. However, the increases in blood sugar in the afebrile and febrile dogs were so nearly alike that the presence of hydremic plethora in one group of dogs and not in the other seems rather paradoxical, especially in view of the claims that the increase in dextrose is one of the chief factors in the blood dilution. From this it seems that the absence of temperature changes after salicyl in normal dogs has some explanation other than the absence of blood dilution. In his study of sodium salicylate, acetylsalicylic acid, quinine, and some other antipyretics on the excretion of nitrogenous constituents in dogs and rabbits, Morinaka found only insignificant variations in these products, and, therefore, no important depression of tissue metabolism. Dreser also found acetylsalicylic acid to be negative in rabbits.

On the other hand, several investigators have demonstrated definite effects on normal temperature in animals. Chirone and Petrucci (195) found small doses of sodium salicylate to lower, and large doses to increase, the temperature in dogs and rabbits, these latter results agreeing with certain ones of Barbour and Herrmann who used doses corresponding to the full therapeutic. Using a special metal cannula through which hot and cold water were passed for stimulation of the heat and cold centers in the rabbit brain, Hashimoto (393) demonstrated a diminution in the irritability of the heat centers after administration of sodium salicylate and antipyrine. The application of

heat alone by this method results in an increase in body temperature and flushing of ears, shivering being absent, while the opposite effects occur on the application of cold. Salicyl diminished the responses to application of heat, and the application of cold removed the diminution. Morphine diminished the responses to both heat and cold, indicating a difference from salicyl, which appeared to have a specific action on the heat centers. These results indicate some central depression from salicyl.

An increase in heat dissipation amounting to 43.3 per cent together with an increase of 39.6 per cent in heat production in normal dogs was reported by Wood and Reichert (394) after doses of from 1 to 5 grams of sodium salicylate. Increased dissipation was regularly observed by Stuehlinger (395) in guinea pigs receiving sodium salicylate. Livon (396) found the carbon dioxide production generally increased in guinea pigs, turtles and frogs with sodium salicylate. In the experiments of Singer (213) on rabbits, 0.3 gram acetylsalicylic acid by mouth decreased the oxygen consumption 17 and 14 per cent, while a "toxic" dose of 0.9 gram increased it 9 per cent. In an extensive and controlled study of the temperature and heat in mice according to modern methods and with the aid of a thermoelectric needle, Yoshinaga (397) observed that 0.3 gram per kilo of sodium salicylate or acetylsalicylic acid orally lowered the temperatures of the peritoneum and the skin of the back. The reduction amounted to about 0.2°C. in the majority of mice with a return to normal at the end of one hour. A detailed study of the mechanism of temperature reduction in a mouse of 12 grams kept at room temperature (18°C.) showed that during a reduction of 2.8°C., there was a greater reduction, i.e., 17.3 per cent, in heat formation (carbon dioxide per hour) than in heat dissipation, i.e., the calories per hour were reduced only 2 per cent. The total calories were reduced from a normal of 200.2 to 196.1 after the salicyl compounds. The distribution of the caloric reduction was as follows: by evaporation, from 16.3 to 15.5, by air, etc., from 0.7 to 0.6, and by radiation and conduction from 183.2 to 180.0. The effects of salicyl were similar to those of other coal-tar derivatives, namely, antipyrine and phenol. In their study of antipyretics on tissue respiration by means of succinhydrogenase, Nitzescu and Cosma (398) observed that sodium salicylate stood next to quinine as a depressor of oxidative

processes. Yamamoto (399) reported that although acetylsalicylic acid possessed a greater antipyretic efficiency than salicylic acid, yet no appreciable difference was found in the combining efficiency of the two compounds with brain tissue.

In rabbits and dogs, the hypodermic injection of cinchophen was found by Starkenstein and Wiechowski (230) to cause some lowering of normal temperature. The action appeared to be both central and peripheral. Central depression was indicated by a diminution in the irritability of the respiratory center, and by a reduction of central vagus stimulation and of asphyxial reactions. The peripheral action was apparently concerned with vasodilation. In some later experiments, Starkenstein (400) found that cinchophen did not lower the temperature of decerebrated and warmed rabbits, but lowered it when the rabbits were removed from the warming box. From this it was concluded that the temperature lowering action of cinchophen must be central, the depression or paralysis of the heat centers impairing heat regulation similarly to the action of antipyrine. Arguing from certain experimental results, namely, that withdrawal of calcium causes fever and increases the irritability of the sympathetic nervous system, Starkenstein sees in this an analogy to the action of cinchophen and some further considerations incline him to the view that the actions of cinchophen are fundamentally concerned with the sympathetic nervous system. However, several other actions do not fit perfectly into this analogy. On the other hand, the lowering of temperature after "leukotropin" (a solution of cinchophen in hexamethyleneamine) in rabbits is attributed by Ullmann (283) to a stimulation of the parasympathetic nervous system. It is claimed that the respiratory center was paralyzed and the pupil constricted simultaneously, the constriction being relieved by atropine. The claims that cinchophen exerts its effects on body temperature through the sympathetic and parasympathetic nervous systems do not seem well established. There is no reason to believe that cinchophen acts differently from salicyl on body temperature, but further and more critical analysis is desirable.

What appears to be a potentiation of cinchophen on temperature in rabbits has been reported by Dittrich (401). Weak and inactive doses of cinchophen caused a marked lowering of temperature when the

drug was injected together with cyanamide, which checks oxidation-reduction processes in vitro and in vivo. This has been confirmed by Hesse (402), who reported that the collapse was so marked that death resulted in the majority of rabbits. Inhibition of catalase, of reduction by different organs and of oxygen consumption by the cyanamide has been demonstrated.

Normal and afebrile men. In 23 afebrile human subjects, Riess (403) observed that doses of 5 grams of salicylic acid lowered the temperature about 0.8°C. in from 4 to 6 hours, the pulse remaining unchanged. Riegel (404) noted practically no change in temperature in human subjects after doses of 5 grams. Gedl (405) made careful observations (after dosage of 5 grams each) in eight afebrile patients by taking the temperature in the axilla and per rectum 5 times daily for several days before the sodium salicylate was administered. Four of the patients showed a small though distinct lowering of temperature; 3 gave variable results, and 1, no response. Moeli (406) noted a fall in temperature in 40 minutes after administration of sodium salicylate; the pulse fell regularly with the temperature, and sometimes there was typical dicrotism. Hare (407) found salicylic acid to reduce slightly the normal body temperature.

In the experiments of Denis and Means (201) on 3 convalescent subjects at rest, there was no demonstrable effect on basal metabolism and respiratory quotient after doses of from about 15 to 23 grams of sodium salicylate. The Benedict universal respiration apparatus with spirometer and mouth piece were used in this work. Using the Benedict respiratory chamber and determining the changes in carbon dioxide excretion and rectal temperature, Barbour and Devenis (408) found that 1 gram doses of acetylsalicylic acid in normal human subjects caused a definite though small increase in carbon dioxide output and heat production amounting to about 6 per cent at the end of $4\frac{1}{2}$ hours. The pulse rate fell at the rate of one beat per minute every half hour. Sleeping subjects showed the same changes. There was, therefore, an increase in basal metabolism, but heat dissipation was not altered, and temperature depression was not demonstrable by this method to an important degree.

Experimental fever. Here salicyl appears to have a more marked tendency to lower the temperature than in health. In fever with sep-

ticemia in rabbits produced by the injection of putrid fluids (blood, etc.), Fürbringer (53) observed that from 0.05 to 0.2 gram of salicylic acid lowered the temperature from a fraction to a degree within $1\frac{1}{2}$ to 8 hours. A complete disappearance of fever occurred in the nine animals observed. The results obtained by Zimmermann (409) were not so favorable as those of Fürbringer. Salicylic acid (0.1 gram) given by mouth or subcutaneously to rabbits previously injected with decomposed urine, and in whom fever and septic processes resulted, lowered the temperature from 0.2 to 0.4°C.; later the temperature increased again, and the control animals died. In other rabbits, the fever produced by injection of putrid blood was reduced with initial doses of 0.1 gram of salicylic acid. The temperature rose again, however, and subsequent doses failed to influence it. According to Hare (407) salicylic acid had no influence on pyretic temperature.

Bondi and Katz (142) claimed that acetylsalicylic acid was antipyretic with puncture fever, while salicylic acid was not, acting in this respect like the antipyrine group. In dogs made febrile by injections of *B. coli*, acetylsalicylic acid and sodium salicylate in doses of from 0.25 to 0.67 gram per kilo were found by Barbour and Herrmann (391) to cause a lowering of from 0.2° to 1.2°C. in temperature. This was accompanied by hyperglycemia and dilution of the blood, the hyperglycemia being quite as marked in the normal dogs though unaccompanied by blood dilution. The intravenous injection of dextrose alone in febrile dogs also reduced the temperature. The hydremic plethora, which resulted from the hyperglycemia after salicyl, occurred only in the febrile dogs, and was responsible for the temperature reduction. The hydremic plethora, it is explained, promotes heat dissipation by radiation and surface evaporation, thereby causing the reduction in temperature. Quinine and antipyrine acted qualitatively the same way as salicyl, though quantitatively differently. A factor which appears not to have been considered in these experiments is renal injury which has been demonstrated to occur after large doses of salicyl. Moreover, any renal injury from the febrile condition would tend to be aggravated by the salicyl, and such marked injury in turn might cause water retention and blood dilution. A moderate degree of renal injury occurring from salicyl in health would not necessarily result in sufficient water retention to cause blood dilution. This was

the case with the afebrile and practically normal individuals of Hanzlik, Scott and Reycraft (163).

Whether the changes in blood sugar and dilution are of sufficient magnitude to explain the action of antipyretic drugs is a matter that can not be considered further here, but would seem to merit further study before it can be accepted without question. The blood changes may be only incidental to the temperature changes. However, anyone interested may consult a recent review of the subject by Barbour (410).

Using dogs rendered febrile with injections of typhoid, paratyphoid and *B. coli* vaccines, Barbour and Lozinsky (299) compared the antipyretic and toxic effects of acetylsalicylic acid, cinchophen and neocinchophen. Acetylsalicylic acid acted more quickly than cinchophen and neocinchophen. However, neocinchophen was the least toxic of all. The ratios of the minimal antipyretic to the minimal lethal doses

$$\frac{C}{T} = \frac{\text{minimal antipyretic dose}}{\text{minimal lethal dose}}$$

were estimated to be as follows: neocincophen $\frac{1}{150}$, cinchophen $\frac{1}{63}$ and acetylsalicylic acid $\frac{1}{25}$. Accordingly, the therapeutic range of neocinchophen was thought to be much greater than that of cinchophen and acetylsalicylic acid. Unfortunately, however, the febrile temperature in dogs with vaccine fever tends to recover spontaneously rather promptly, and frequently additional injections of the vaccine fail to produce fever. In other words, the error in such experiments may be considerable, and the antipyretic action due to spontaneous recovery and not to the drug administered. Extensive series of experiments with adequate controls are desirable in making comparisons of the efficiency of different drugs. Since neocinchophen is so poorly absorbed (discussed under absorption) from the alimentary tract, it is conceivable that the administration of an equivalent quantity of inert material, such as charcoal or kaolin, might have produced the same result in Barbour and Lozinsky's experiments. The comparative weakness of neocinchophen as an antipyretic in rheumatic fever was indicated by the results of Hanzlik, Scott, Weidenthal and Fetterman (291).

Heat regulation in clinical fevers. No reduction of carbon dioxide in febrile subjects with typhoid, tuberculosis and diphtheria was observed by Buss (389) after doses of from 6 to 8 grams of sodium salicylate. Frequently there was an increase in the carbon dioxide, but no diminution was observed in normal subjects. However, his method was probably not sufficiently accurate. In carefully controlled experiments, and using the Benedict respiration chamber, Barbour (411) made observations on 5 febrile, temporary febrile and convalescent subjects suffering with tuberculosis, osteomyelitis and empyema receiving 1 gram doses of acetylsalicylic acid. There was a marked antipyretic action which was not exhibited in normal subjects. In $1\frac{1}{2}$ hours the temperature fell 0.81°C. in 6 experiments on 4 subjects as against an average rise of 0.18°C. on 4 control days. After the administration of the acetylsalicylic acid, the heat elimination increased 38.2 per cent, and the antipyretic effect was due mainly to this change, as with other coal-tar derivatives except possibly antipyrine. The fall in temperature was accompanied by a decrease of 3.5 per cent in heat production as compared with control days, due merely to cooling of the body. The drug caused an average decrease in pulse rate of 10 beats per minute, and some cardiac disturbance was noted in 2 out of the 5 subjects. This suggests some circulatory depression. The return to the initial temperature was brought about essentially by a reduction of heat elimination. Acetylsalicylic acid appeared to increase the respiratory quotient in these antipyretically sensitive individuals, which however was not the case in normal subjects. The sensitivity was not concerned with "stimulation" of a "depressed" heat regulating mechanism or due to lack of combustible material (dextrose).

Zimmer (412) administered cinchophen, maretin, pyramidon and lactophenin in small doses during four to five days to moderately febrile patients with sepsis, grippe, and tuberculosis and observed the effects on the concentration of blood proteins refractometrically. The body weight generally tended to increase with the dilution of the blood, and to decrease with the increase in blood concentration. In other words, retention of water occurred as the result of the administration of the antipyretics, and the mechanism of the retention was ascribed to an increase in capillary permeability. The mechanism was partly

sustained by certain effects of cinchophen on blood dilution and body weight after intravenously injected 10 per cent sodium chloride solution. However, the results of this author do not appear critical. The changes reported were small and inconstant, and the renal factor, that is, diminution in functional efficiency, was not considered. As far as blood dilution goes, the results with cinchophen and the other drugs used are in agreement with those of Barbour and his associates with salicyl. It will be recalled that Barbour attributes the blood dilution of antipyretics to the accompanying hyperglycemia, whereas Zimmer attributes it to altered capillary permeability, the latter being rejected by Barbour in his consideration of various possible mechanisms. According to Zimmer, v. den Velden, in 1912, was the first to recognize a relationship between antipyresis and water content of the blood.

ANTIPYRETIC ACTION IN MISCELLANEOUS CLINICAL FEVERS

The use of salicyl in the form of a decoction of willow bark (most species of the large genus *Salix*) as a general febrifuge dates from ancient times. The practice continued through the medieval and up until modern times. During the Napoleonic wars willow bark was used successfully as a substitute for cinchona bark. The active constituent of willow bark is the bitter glucoside, salicin ($C_{13}H_{18}O_7$), which on hydrolysis yields dextrose and saligenin, or salicyl alcohol, ($C_6H_4(OH)\cdot CH_2OH$), and from which salicylic acid can be prepared. However, salicin, though effective as an antipyretic providing enough is administered, is now obsolete as a remedy because of the more easily obtained, economical and dependable synthetic salicyl compounds. For this reason it is not considered in this review, but a number of references have been appended in the bibliography for those who may be interested.

Until 1874, quinine was the antipyretic in general use for the treatment of all sorts of febrile conditions, but after that date the salicyl compounds came into use and were quite as extensively employed as quinine with equally good results. The following summary will testify to their efficiency in a variety of fevers. Their use in rheumatic fever will be considered in the next section.

Sodium salicylate was first introduced into clinical therapeutics by

C. E. Buss of Basel in 1875 (389). He demonstrated its antipyretic action in many patients with typhoid, tuberculosis, diphtheria and in four patients with rheumatic fever. The dosage used ranged from 6 to 8 grams. Buss' results were quickly confirmed by numerous clinicians throughout the world. The occasional failures were due to inadequate dosage. Wolfberg (413), for instance, found that 4 grams or less of salicylic acid was feeble and uncertain as a general antipyretic as compared with similar doses of quinine. Pel (414) reported that the majority of his thirteen cases of intermittent fever were benefited by 4 to 16 gram doses. According to Fehling (91), fever accompanying the puerperium with foul lochia was reduced and the lochia became less putrid when 4 to 8 daily vaginal douches of 1:1000 to 1:600 of salicylic acid were given.

Good results in typhoid fever (9 cases) were reported by Nathan (415) after doses of 8 grams as the first dose and of half that much 2 hours later. At the same time the pulse rate fell to normal, and the strength was somewhat increased. Riegel (404) found the antifebrile effects of salicylic acid in typhoid more pronounced than with quinine, but the fever recurred and no effect on the duration of the disease was noticeable. Johanssen (416) reported that about one-half of his 15 cases of intermittent fever were rendered afebrile after doses of 1.5 to 3 grams given at short intervals; no effects were noted in hectic fever after 2 gram doses, there being a fall usually of 0.1°C. Hiller (417) stated that he obtained favorable results in seven cases of intermittent fever after 12 to 28 grams of salicylic acid. In 3 cases in which the acid was given per rectum, pain and diarrhea were observed. In 23 cases of typhoid reported by Fischer (418) in which the temperature was at 39.5°C. and doses of 1 to 3 grams of the salicylate or the acid were given in the evening, the temperature was reduced by morning. In a study of more than 400 cases of different febrile conditions, Riess (403) found that daily doses of 5 grams of salicylic acid lowered the temperature 2, 3, 5 and 6 degrees, and often in 1 to 2 hours. The more intense the fever the smaller and shorter the action. In 260 cases of typhoid, of which 209 were recent, and in which the temperature was at 39°C., the first dose had no effect, but later the temperature fell, and 8 to 10 doses of salicylic acid served to keep the temperature at normal. The pulse rate remained uninfluenced, but

often became stronger and showed an inconstant dicrotic notch; vomiting and signs of collapse were rare. The course of typhoid fever with salicylate treatment was shortened to an average of 13.1 days in 164 cases. Antifebrile action was also demonstrated by Riess in the following acute diseases; croupous pneumonia, erysipelas, scarlet fever, rheumatic fever, and chronic phthisis. Butt (419) found 4 to 8 grams of salicylic acid to reduce the temperature in typhoid, erysipelas and rheumatic fever. Moeli (406) reported a fall of 1.5 to 3°C. after comparatively large doses within from 4 to 16 hours in typhoid. This was accompanied by marked sweating. Rectal administration required larger doses; subcutaneous injection gave prompt relief. Rosenthal (420) observed no reduction of temperature in typhoid and intermittent fevers; in pneumonia a fall of about 0.8°C. occurred. Hildebrandt (421) and Jahn (422) observed the fever to be lessened and the duration of the disease shortened in typhoid. Thomas (423) thought the antiperiodic action of salicylic acid was greater in malarial than in rheumatic fever, a failure being noted in only 3 out of 100 cases of intermittent fever. Raymond and Schultz (424) reported that the fever and general symptoms of scarlet fever subsided after the administration of 6 grams daily of sodium salicylate during 3 days, and complications were prevented.

Bondi (425) found that 0.25 gram of acetylsalicylic acid was antipyretic in typhoid. The antipyretic responses reported by Barbour (411) with acetylsalicylic acid in tuberculosis, osteomyelitis and empyema, and by Zimmer (412) with cinchophen in sepsis, tuberculosis and grippe, have been referred to in the preceding section.

The principal deduction from the results in this section is that the salicylates can act efficiently as antipyretics in clinical fevers in general, irrespective of their etiology, which suggests that their antipyretic action in rheumatic fever is not specific. The same holds for cinchophen.

ACTIONS IN RHEUMATIC FEVER

Probably the most striking clinical action of salicyl and cinchophen is the prompt and complete relief of all the symptoms of rheumatic fever. This includes the fever, the immobility of, and the pain, redness, swelling and effusions in, the joints, the general discomfort,

and the accelerated pulse and respiration. The efficiency of salicyl is so good that some have regarded it as a diagnostic and specific agent for this condition. Curiously enough cinchophen has not been so regarded, though there is no reason to believe that it acts differently from salicyl. The various aspects of the actions of these drugs in rheumatic fever may now be considered with the aim of arriving at some idea of the mechanism of their antirheumatic action.

General and historical

Although Buss (389), Riess (403) and Butt (419) demonstrated the antipyretic action of sodium salicylate in rheumatic fever, they laid no stress on the other beneficial effects that were obtained at the same time, and failed to recognize its great therapeutic value in this condition. The credit for having first recognized the full therapeutic response to be obtained with salicyl in rheumatic fever must be given to Stricker of Traube's Clinic in Berlin in 1876 (426). The observations of Stricker were confirmed during the same year by Katz (427). Stricker regarded salicylate as a radical agent and a specific cure for rheumatic fever (O. Meltzer (428)).

In Petit's contribution (429), the earlier reports on clinical cases by many clinicians are reviewed, and the beneficial effects of salicin, sodium salicylate and salicylic acid discussed. Broadbent (430) obtained beneficial results in rheumatic fever after 7.5 to 20 grains (0.5 to 1.3 grams) every hour. The pulse rate fell before the temperature, and the rheumatic pain disappeared in 4 days after the treatment was begun; the cardiac phenomena remained unchanged. Schumacher (431) stated that a complete cure in rheumatic fever may be obtained within from 12 to 48 hours, but that in a few cases there was recurrence of the disease. Steinitz (432) observed that salicylate gave a more prompt relief in rheumatic fever than other methods of treatment (injection of phenol, etc.); chronic rheumatism was not benefited. G. Sée (170) obtained best results in rheumatic fever. In 12 to 18 hours there was a fall of temperature, and the joint pains disappeared so that at the end of 3 to 4 days the patients could walk. Sée observed no antipyretic effects in typhoid and intermittent fevers. V. Ibell (433) observed most marked benefits in rheumatic fever after hourly doses of 0.6 gram of the sodium salicylate; the total duration

of the disease being 3 to 31 days; recurrence occurred in about 20 per cent of the cases. The drug was inactive in gonorrheal rheumatism, and other pyemic joint affections. Oetinger (434) found that recurrences and complications rarely occurred when salicylate was used. Cavafy (435) observed that pain in rheumatism was relieved in about 2 days (average). Vulpian (436) observed that from 4 to 8 grams per day in the young and adults gave relief in rheumatic fever within 48 hours. Rheumatic pleuritis, peritonitis, and encephalopathy were not influenced by salicylate. Pain in rheumatic fever, muscular rheumatism, rheumatic neuralgia, tabes dorsalis and certain cephalalgias were thought to be sometimes eased by sodium salicylate, although not generally. No influence on sensation was observed by Vulpian. In Hall's (437) cases, pain and fever in rheumatism were relieved more quickly with salicylate than without the drug. In a statistical study of results obtained with salicylates in rheumatic fever, Reihlen (438) found that 95.2 per cent of cures were effected in men; 91 per cent in women. A combination treatment of salicylic acid with salol in which the salol predominated was successfully used by Aufrecht (439) in rheumatic fever. About the only failure with salicylates in rheumatic fever was reported by Fraser (440). This occurred in patients with inflammation of the genito-urinary passages. It was explained as due to lack of tolerance of the salicyl owing to lessened elimination in renal disease, for whenever albuminuria was present, symptoms of salicylism occurred. Strictly speaking this was not a therapeutic failure of the salicyl, and, therefore, it can be said that the earlier observers unaniminously agreed on the full therapeutic benefits to be obtained from the drug in rheumatic fever. This has been amply confirmed by five decades of steady usage in the treatment of this condition, and in many investigations. Even the necessity of using large doses was appreciated very early.

Symptomatic effects of the different salicyl compounds

The most useful criterion of therapeutic response in rheumatic fever is the antipyretic action. With full therapeutic doses of sodium salicylate the temperature falls rapidly to the normal level, and with it the pulse and respiration are slowed and the pain dis .ppears. The pain is lessened almost at once, but complete mobility of the joints is

usually not restored until some time after the full dosage has been administered. An early subjective recovery is frequently observed so that the patient may feel so well as to be able to move around and even leave the bed and walk on completion of the administration. In a statistical study of over 400 rheumatic patients by Hanzlik (476), complete relief of all symptoms occurred in 81.8 per cent of males and females and complete failure in only 1.6 per cent receiving sodium salicylate. In some patients the temperature becomes subnormal; in others it remains slightly febrile, leaving the patient uncomfortable. This may be due to spreading of the infection or to insufficient dosage of the drug, but sometimes a complete recovery is not obtained in spite of large doses. With the majority of patients a single course of medication, giving 1 gram doses of the drug hourly to the point of salicylism, suffices. Then the medication may be stopped, though it is the practice with many to continue the medication with small doses 2 or 3 times daily for some time afterwards as a routine. Continued medication is always indicated if the fever continues or there is an extension of joint involvement, the latter being indicated by the rise in temperature.

The lack of promptness or completeness, or both, of the response to salicyl has been taken by some to indicate the presence of some other form of arthritis or disease than rheumatic fever. However, the diagnostic significance of such a result may be questioned, for more specific remedies than salicyl, such as quinine and arsphenamine, sometimes fail to give the desired results in their own fields, that is, in malaria and syphilis. The explanation of the lack of response in occasional cases of rheumatic fever is not at hand, though it probably falls within the range of variations of biological reactions in general. Nevertheless, the therapeutic response to salicyl in the vast majority of patients with rheumatic fever is prompt and definite and probably more dependable than the therapeutic response to many other drugs in other diseases. Figure 7 illustrates typical antipyretic responses of eight patients suffering with rheumatic fever reported by Hanzlik, Scott and Gauchat (441). The results were obtained under controlled conditions and the patients received no other medication besides sodium salicylate. The majority of the patients were kept for two days without medication; then on receiving salicyl to toxicity (salicylism) the temperature

fell to about the normal level at the end of 8 to 10 hours. The majority of the patients responded completely to a single full therapeutic dose, only two requiring repeated courses of salicyl medication, and in only one other patient did the temperature fall somewhat more slowly than usual. The mechanism of the antipyretic action has been discussed in the two preceding sections.

The effusion into and swelling of joints disappear some time after the pain and fever. This might be expected because absorption of the fluid is a relatively slow process. No accurate data on the time of recovery

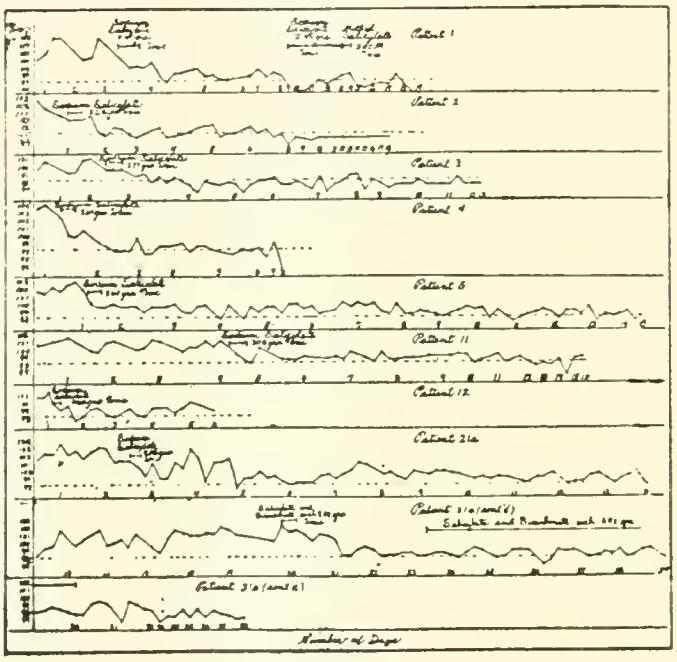


Fig. 7. Typical Antipyretic Responses to Full Therapeutic Doses of Soduim Salicytate in Rheumatic Fever

are available, but, in general, recovery may be apparent or even complete at the end of 24 hours, more definitely at the end of the second to third day after a full therapeutic dose of the drug. There is considerable variability. Complete rest facilitates recovery. Moreover, the joint symptoms tend to recover spontaneously without salicyl medication as was demonstrated in the study of Hanzlik, Scott and Gauchat (441). Therefore, it may be doubted if salicyl per se facilitates the recovery of the objective symptoms in the joints independently of rest and spontaneous recovery. However, there seems to be no doubt that the relief of pain, tenderness and "soreness" when movements are

attempted is due directly to the drug. From his study of a large series of cases, Swift (442) concludes that the distressing symptoms of arthritis are easily controlled by either salicylate or neocinchophen; that the exudative features in rheumatic fever are less prominent under the full influence of salicyl than without the drug and that the disappearance of exudation and the symptoms after salicyl is the most characteristic feature of the disease. The subcutaneous nodules may appear continuously while the patient is receiving salicyl.

On the other hand, the recovery of the accelerated pulse and respiration is mainly the indirect result of defervescence of the fever. This is the tendency in spontaneous recovery from fevers in general. Swift (442) reports a slowing of 20 to 30 per cent in pulse rate. In the section on circulation, it was seen that full therapeutic doses of salicyl may accelerate the heart in normal individuals due probably to diminished peripheral resistance owing to the relaxation of peripheral vessels, and the respiration is simultaneously accelerated, if anything. The relative slowing of the pulse and respiratory rates in rheumatic fever is made apparent by removal of the cause (fever) of the marked increase in these functions. The changes in these functions do not have an important bearing on the general symptomatic relief in rheumatic fever.

The marked sweating of the disease is temporarily aggravated by medication with salicyl, but this only facilitates the defervescence of the fever, and therefore, recovery. The efficiency of nature's protective mechanism appears to be improved by the drug. The sweat vesicles are uninfluenced, and whether the composition of the sweat is affected or not is unknown. Unfortunately, speculations in text-books on the possible changes in the alleged marked acidity of the sweat in rheumatic fever lack a scientific basis.

Whether, or to what extent, the amelioration of the symptoms of general discomfort, that is, the subjective sensation of aching, weakness and stiffness in the limbs, restlessness, etc., should be attributed directly to salicyl is debatable. There is no doubt that medication with antipyretics and analgesics, whether of the salicyl or non-salicyl type, makes patients decidedly more comfortable than no medication at all. However, the experience of comfort during medication may be only the result of the relief of fever and pain.

As to duration of the disease under treatment with salicyl, this varies with the initial severity of the attack, multiple extension to joints, the individual and other factors. The mean duration in 8 patients of the series of Hanzlik, Scott and Gauchat (441) was 15 days (range 6 to 38 days), which was 9 days shorter than the duration of illness in patients on non-salicyl medication. J. L. Miller (443) found that the period of stay in hospital in treated and untreated patients did not differ. Statistics on the subject are unreliable, being subject to the individual opinion of the physician as to what constitutes a desirable period of rest. The end point is not always sharp. Swift (442) thinks that the period of rest should be as long as signs of infection persist, but the latter is not always interpreted by the same criterion; and cultures of the infective agent are precluded.

It is the general experience that treatment with salicyl alone from the beginning insures freedom from symptoms of rheumatic fever in the majority of patients. Accurate data, however, on the recurrence of symptoms are limited. In the statistical study by Hanzlik (298), about 18 per cent of the patients were incompletely or not at all relieved by the administration of sodium salicylate in full therapeutic doses, and in the large majority that was relieved, it was certain that salicyl was repeatedly administered to many patients because of recurrence of the symptoms. Two out of the 8 rheumatic patients in the series of Hanzlik, Scott and Gauchat (441) required repeated salicyl treatment for recurrence. J. L. Miller's (443) statistics showed that the salicyl treated patients relapsed more frequently than the untreated; that the freedom from pain became earlier in the treated, but that the duration of pain in the treated was no different from that in the untreated. Swift (442) found that relapses were often heralded by a declining weight so that body weight was a good index of recovery. The body weight might still fall during treatment with salicyl when other symptoms had been controlled. Swift, Miller and Boots (284) found the leukocytic curve of considerable value. That is, in the absence of evidence of concomitant non-rheumatic infection, persisting leukocytosis signified persistence of rheumatic infection, and, conversely, repeated normal counts indicated that the attack was drawing to a close, the latter being true providing the patient was not receiving salicyl medication. When the leukocytes

remained normal on discontinuance of the drug, no relapse occurred and the patient recovered. When the drug was stopped and the leukocyte count increased to 4000 or more above the previous level, this showed that the infection had not stopped.

The derivatives of salicyl, namely, acetylsalicylic acid, salicyl salicylate and methyl salicylate, act qualitatively the same way as sodium salicylate in rheumatic fever. Their use in this condition has been discussed in general papers by Gordon (444) and Klaveness (445). Quantitatively, there are some differences from sodium salicylate. From a statistical study of clinical dosage by Hanzlik (298), methyl salicylate was found to be about as efficient as sodium salicylate, but acetylsalicylic acid was about $1\frac{2}{3}$, and salicyl salicylate, about twice, as efficient. Conversely, the effective dosage of these latter derivatives would be $\frac{2}{3}$ and $\frac{1}{2}$, respectively, of that of sodium salicylate. Beneficial effects from acetylsalicylic acid in rheumatic fever were observed in connection with the study of its excretion by Hanzlik and Presho (21). About the only advantage acetylsalicylic acid and salicyl salicylate possess is their more pleasant taste, but this does not mask the central nausea and vomiting of the "toxic" dosage. Methyl salicylate is unpleasant by mouth and its absorption is irregular and somewhat uncertain (discussed under absorption). Local application of the ester to joints is sometimes used with the idea of securing absorption and avoiding nausea, etc., but this notion is erroneous, since the ester is not sufficiently absorbed in this way to secure the systemic effects of salicyl. Any benefits from local application are due entirely to counterirritation. From 10 to 16 grams of novaspirin (anhydromethylenecitryl salicylic acid) were found by Hanzlik, Scott, Weidenthal and Fetterman (291) to be without demonstrable benefit in 3 patients with rheumatic fever. This was due to poor absorption, which amounted to a median of only 17.3 per cent of the total administered.

Effects of cinchophen and derivatives

These are precisely the same as those of salicyl just discussed and the details need not be repeated. However, some comparisons that have been made with salicyl compounds may be of interest.

It appears that Oeller (446), under Strümpell at Leipzig, in 1911,

first reported on the beneficial effects of cinchophen in rheumatic fever. A further report was made by him in 1912 (447); the best results were obtained with 3 grams given within 6 hours, when there was a rapid disappearance of redness and swelling in joints, marked sweating and prompt antipyretic action. Oeller thought cinchophen was superior to acetylsalicylic acid because the latter did not lower the temperature as promptly as, and left more cardiac side actions than, cinchophen, but it may be doubted if the dosage of acetylsalicylic acid was adequate. In the same year, Heller (290) reported that only from 25 to 50 per cent of his rheumatic patients obtained partial or temporary relief, but this lack of success appears to have been due to inadequate dosage. The subsequent reports by Bendix (448), Neukirch (449), Klemperer (450), Jokl (451), Hahn (452), and Beeck (453) on the symptomatic relief in rheumatic fever were more favorable although certain claims of superiority over salicyl by some of these authors have not been supported by later work. This statement pertains to the claim of Bendix (448) that undesirable side actions do not occur, and that of Friedberg (454) that renal irritation could be avoided with cinchophen. Neukirch (449) compared cinchophen with melubrin and acetylsalicylic acid in 8 cases of rheumatic fever and claimed that cinchophen lowered the temperature after the other 2 drugs failed. After cinchophen, the fever disappeared at the end of 48 to 60 hours, and pains in joints together with the exudation and erythema at the end of 12 to 24 hours. Out of 31 patients, 28 responded completely to cinchophen, while 2 patients, who failed to respond to cinchophen, responded to salicylate. The recurrences were no greater after cinchophen than after salicyl. Jokl (451) reported complete benefits, that is, antipyresis, analgesia, and disappearance of local heat and swelling, in 25 out of 30 rheumatic patients treated with cinchophen, and, in some, the drug acted when salicyl did not. Klemperer (450) reported successful results with cinchophen in 300 cases of rheumatic fever, and suggested that the benefits rested on a complicated antiphlogistic-analgesic action similar to that of salicyl.

In the 11 patients treated with cinchophen and neocinchophen, the therapeutic responses observed by Hanzlik, Scott, Weidenthal and Fetterman (291) were complete so that direct comparison with salicyl in the same patients was precluded, but an idea of their comparative

efficiency was obtained from the dosage of salicyl previously established. Partial symptomatic relief was obtained with doses of from 3 to 6 grams; complete relief with 10 to 13 grams of cinchophen, and with 11 to 16 grams of neocinchophen. The partial relief that was obtained by less than "toxic" doses of both cinchophen and neocinchophen indicated no special advantage over salicyl, since the same holds true of salicyl compounds and other analgesics. The dose of cinchophen was about equal to the full therapeutic dose of sodium salicylate, and, therefore, the therapeutic efficiency was about the same, while neocinchophen was less efficient, since larger doses of it were necessary to secure a complete therapeutic response. This was because of its low solubility and absorbability, which was also responsible for the small amount of gastric irritation as compared with cinchophen, the latter causing more distress than sodium salicylate. The symptoms of salicylism were about the same and renal injury somewhat less after cinchophen, and both were less pronounced after neocinchophen than after sodium salicylate in corresponding doses. The cardiac slowing produced by cinchophen was presumably due to direct circulatory depression, since it occurred in both febrile and afebrile subjects. The antipyretic action of cinchophen was more prompt and marked than with neocinchophen despite the higher doses of the latter. The diaphoresis, which occurred simultaneously, was marked with both drugs. Reduction of the fever to a subnormal level of temperature was demonstrable in the majority of patients at the end of 8 to 12 hours with the dosage of cinchophen used, but with neocinchophen it took about 24 hours for the temperature to approach the normal, though it tended to remain somewhat above the normal level. All this indicates that neocinchophen possesses a slower and less perfect antipyretic action than cinchophen and salicyl. Typical antipyretic responses of cinchophen and neocinchophen in rheumatic fever are illustrated in figures 8 and 9, respectively. Chace, Myers and Killian (293) observed a greater antirheumatic efficiency with neocinchophen than with salicyl, because the pain and swelling of joints in several patients yielded to neocinchophen after a failure to ameliorate these with salicylates, but, in general, there were small differences in the analgesic, antipyretic and uric acid elimination effects of these drugs. Barbour, Lozinsky and Clements (292) found neocinchophen fully

efficient in 12 patients with rheumatic fever, the total dosage required being from 10 to 16 grams; and 7 to 8 grams gave partial relief. The general absence of gastric symptoms and renal injury inclined them to the belief that the drug can be administered without harmful effects. Miller and Boots (294) reported that neocinchophen gave relief in some cases of rheumatic fever in which the salicylates failed, or the patients were so susceptible to the "toxic" effects of salicyl that enough could not be given. They stated that neocinchophen required

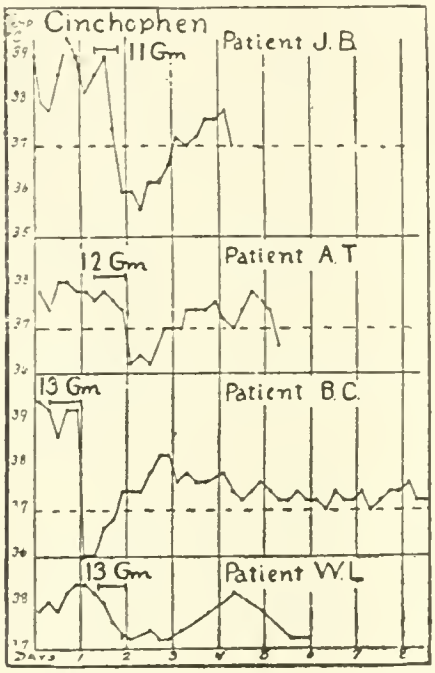


FIG. 8

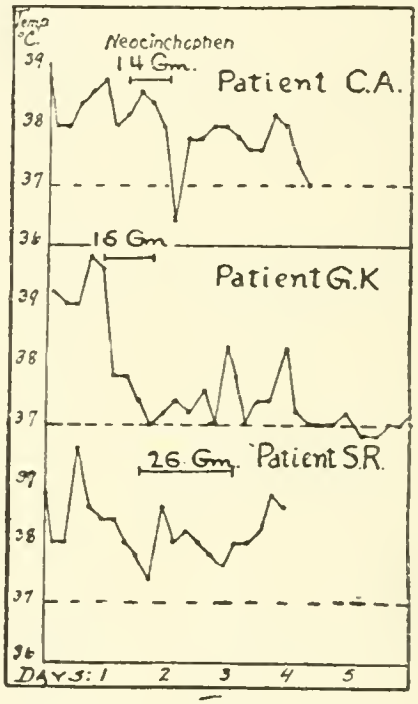
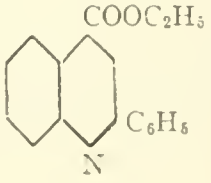


FIG. 9

FIG. 8. ANTIPYRETIC ACTION OF CINCHOPHEN IN RHEUMATIC FEVER

FIG. 9. ANTIPYRETIC ACTION OF NEOCINCHOPHEN IN RHEUMATIC FEVER

large doses, but was less "toxic" and equally or more effective. As an antipyretic, however, it acted more slowly than acetylsalicylic acid, but was more lasting, this being ascribed to slower absorption, which also accounted for its being better borne. Miller and Boots (295) tried the ethyl ester of cinchophen,



in 8 patients with rheumatic fever, using total doses of from 9 to 17 grams during the first 24 hours, and after that, from 4 to 5 grams per diem during variable periods. Full therapeutic responses were obtained in 5 patients, the remaining 3 showing relapses after discontinuing the drug but being relieved by acetylsalicylic acid. In the patients that were promptly benefited, the arthritis, fever, and tachycardia disappeared at the end of 24 hours. The symptoms of salicylism that were present were milder than after salicyl. As an antirheumatic remedy, this ester corresponded closely to neocinchophen, but was probably less effective than salicyl. The advantage of neocinchophen and the ethyl ester of cinchophen over salicyl, that is, the relative absence of side actions, is obviously secured at the expense of relatively poor solubility and absorbability of these drugs. The diminished efficiency, in consequence of these properties, could be corrected to some extent at least by increasing the dosage, but this means that the therapy is less economical and more inconvenient and burdensome for the patient, and, therefore, these drugs do not appear to possess the advantages over salicyl claimed for them.

Cardiac lesions and the heart in rheumatic fever

The lesions accompanying the fever may be located anywhere in the heart. However, the most common lesion is an endocarditis of the mitral valve; next in order, perhaps, is pericarditis. The observation was made by Vulpian in 1880 (436) that these lesions persist unchanged in spite of salicyl medication, and that other visceral manifestations in rheumatic fever are not arrested. In the clinical statistics of Reihlen (438), sodium salicylate was found to be neither prophylactic nor curative for cardiac complications. Cockayne (455) found the cardiac lesions in chorea also uninfluenced. From statistical studies, J. L. Miller (443) concluded that cardiac complications were not less in salicyl treated patients.

An interesting explanation of the greater frequency of endocarditis in the left than in the right auriculoventricular orifice was first suggested by Binz (456) and then by Poullson (139). It was that the carbon dioxide tension in the left heart was too low (only 2.8 per cent carbonic acid, as stated by Binz) for the liberation of free salicylic acid, which was assumed to take place in the right heart in virtue of the

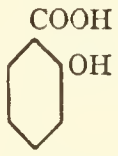
higher carbon dioxide content, and to prevent the growth of organisms there. Binz visualized the mechanism further as follows: He thought the carbon dioxide in the heart was not subjected to the increased pressure which was exerted in the tense fibrous tissues enveloping the joints, and that in the heart the circulation was rapid and unimpeded, while in the inflamed joints the vessels were compressed, and the blood, overcharged with carbon dioxide, flowed more slowly through them. It seemed to him that in the heart the very conditions were wanting under which salicylic acid could be liberated by carbon dioxide, and he stoutly maintained that the sodium salicylate did not circulate in the inflamed parts of the body as the salt without undergoing a change there, and without having an effect on the part. However, so far as the carbon dioxide factor in the liberation of free salicylic acid is concerned, this theory is wrong. For, in the experiments of Hanzlik (137), free salicylic acid was not demonstrable in the slightly acid (pH 6.8) cardiac (right) and arterial bloods of dogs dying of asphyxia, and it is highly improbable that the cardiac blood of rheumatic fever would contain more free carbon dioxide than that of fatal asphyxia. Finally, it was indicated in the section on distribution that a much higher degree of hydrogen ion concentration (acidity) than is compatible with life is necessary for the liberation of free salicylic acid from salicylate in the presence of serum. It was seen also that sodium salicylate is not antiseptic, the concentration of about 0.02 per cent occurring in the circulation after full therapeutic doses being far below any possible bacteriostatic action. Therefore, the explanation of the endocarditic phenomena must be sought elsewhere.

Beneficial effects of both salicyl and cinchophen on the heart are conceivable in other ways. Swift (442) interprets a favorable effect from the reduction of 20 to 30 per cent of the pulse rate due to the salicyl medication. That is, this amount of reduction in the rate reduces the number of impacts of the injured valves by a corresponding amount. The time necessary to keep the patient quiet, Swift believes, is the most important part of the treatment, and the heart naturally participates in the benefits from this period of rest. The abolition of pain, fever and insomnia is an important contributory factor. Swift suggests further that if the edema is eliminated from the valves by the antirheumatic drugs in the same manner as from periarticular tissue,

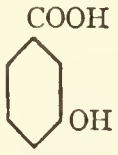
it is conceivable that the endocardium might be spared some traumatic injury. This is at least plausible. The incidence of subsequent carditis in chorea patients was found by Bertram (457) to be lower in those treated with sodium salicylate than in those treated with arsenic, sedatives or rest alone without drugs. He claimed also that salicylate was more effective in reducing carditis accompanying arthritis than in chorea. As far as heart failure in polyarthritis is concerned, Ehrström and Wahlberg (459) found, in an extensive statistical study, that salicyl therapy had no effect on this, nor on the infectious process, and they add that the infection would not be affected because salicyl is not a specific agent for the organism but acts symptomatically.

Chemical structure and composition of salicyl and other agents and antirheumatic action

It is interesting to note that it is only the ortho-hydroxybenzoic acid (salicylic acid),



which gives prompt relief in rheumatic fever. It was shown by Stockman (37) that the meta-hydroxybenzoic acid,

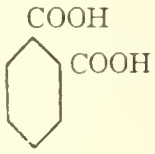


and the para-hydroxybenzoic acid,

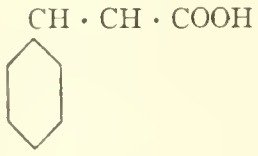
are practically inert as antiseptic and antirheumatic agents. One gram doses two-hourly of these acids up to a total of about 32 grams were ineffective in rheumatic fever, and their fatal doses for rabbits

were about twice that of salicylic acid. These isomers do not give the typical iron color reaction of salicylic acid. This means that the hydroxyl (OH) group has lost its phenolic character chemically. They are, however, conjugated with sulphuric acid in the body as they are excreted as ethereal sulphates, and, therefore, behave physiologically like phenols in general (Sherwin (459)). Stockman (37) thinks that the pharmacological action of salicylic acid depends on the presence of the hydroxyl (OH) and carboxyl (COOH) groups in juxtaposition. No explanation as yet has been offered why the (OH) and (COOH) groups in other positions, as in the meta-and para-acids, do not possess the same action as the ortho acid.

Benzoic acid ($C_6H_5 \cdot COOH$) was found by Stockman (37) to be effective in rheumatic fever and it caused symptoms of salicylism, but the efficiency appeared to be somewhat less than that of salicylic acid, while ortho-phthalic acid,

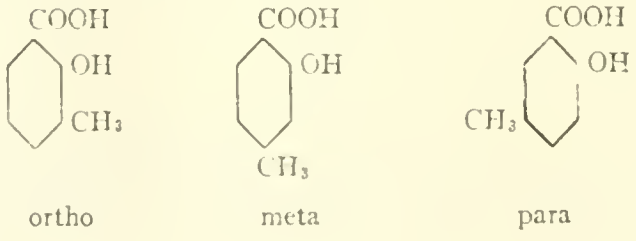


and cinnamic acid,



were inert.

The cresotinic acids ($C_6H_3 \cdot OH \cdot COOH \cdot CH_3$) bear a close chemical resemblance to salicylic acid ($C_6H_4 \cdot OH \cdot COOH$), and have been tried as antirheumatic remedies. Three out of the ten isomeric cresotinic acids are known as the ortho-, meta-, and para-cresotinic acids and have structural formulae as follows:

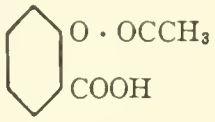


ortho meta para

In all three acids, the hydroxyl (OH) group stands to the carboxyl (COOH) group in the same position as it does in salicylic acid, while the methyl (CH_3) group occupies in reference to the (OH) group the ortho, meta and para positions, respectively. All three acids react chemically with iron giving a purple color, and the sodium salts all were found effective in rheumatic fever by Stockman (460). The para-cresotinate was probably somewhat less efficient than the meta-and ortho-salts. The dosage necessary of all three salts was considerably higher than that of sodium salicylate. This amounted up to 40 grams for the ortho-cresotinate, and was around 32 to 36 grams of the meta- and para-cresotinates. The effects in general were almost identical with those of sodium salicylate. May (461) reported satisfactory results with sodium ortho-cresotinate in rheumatic fever, but Demme (463) decided that the sodium para-cresotinate was less efficient. Stockman (460) quotes Koranyi and Gatti as having found these compounds effective as antipyretics in typhoid, phthisis and pneumonia. In rabbits, May (461) found the lethal doses of the ortho- and meta-salts to be about equal, the para-cresotinate being less poisonous. All this indicates that some modification of the physiological activity of the ortho hydroxy (OH) group in the cresotinates may be produced by changing the position of the methyl (CH_3) group. However, the differences between the different cresotinates, as far as antirheumatic action is concerned, are not marked and they do not offer any advantages over the salicyl compounds.

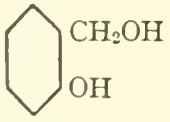
The efficiency of salicyl compounds depends partly on the amount of salicyl which they contain or can develop in the body, and partly on other factors as yet imperfectly understood. The amount of salicyl that may be present or liberated bears on the dosage. That is, the dosage is inversely proportional to the salicyl content. Among the other factors, lipoid solubility, and the methyl, acetyl and salicyl groups play some part. This is illustrated by the relatively higher ($1\frac{2}{3}$) therapeutic efficiency and "toxicity" of methyl salicylate and acetylsalicylic acid than that of sodium salicylate despite the lower (8.6 per cent) salicyl content of acetylsalicylic acid, while that of methyl salicylate is only 5 per cent higher. The therapeutic efficiency of salicyl salicylate is about twice that of sodium salicylate, and the salicyl content, only 20 per cent higher. In the section on excretion,

it was shown that all these compounds circulate through the body unchanged to a large extent, so that their structure is brought into intimate contact with the tissues. Originally, Dreser (11) thought that only those derivatives which would easily liberate salicyl in the tissues would be therapeutically useful, and for that reason discarded the methyl compound corresponding in structure to acetylsalicylic acid,



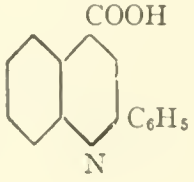
the latter answering the purpose mainly because he found it pharmacologically more active. In this he was not altogether correct because acetylsalicylic acid circulates unchanged. The mechanism of its higher efficiency is not understood, but it may be that the lipoid solubility of the acetyl, and also of the groups of the other derivatives just considered, in some way further augments the action of the salicyl group.

Salicyluric acid ($C_6H_4 \cdot OH \cdot CO\text{-}NH \cdot CH_2 \cdot COOH$), the conjugation product of salicylic acid and glycocoll, was found by Stockman (176) to be ineffective in one patient with rheumatic fever who was given 20 grams. This does not settle it, and actually the temperature was falling under salicyluric medication when sodium salicylate was administered to the patient. As far as is known, Stockman is the only one who has tried this product in rheumatic fever. Saligenin, or salicyl alcohol,

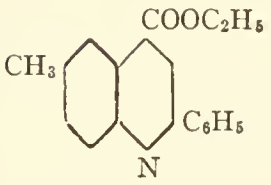


was found by Stockman (176) to be fairly efficient as an antirheumatic in total doses of about 6 grams.

That the hydroxyl group is not necessary, nor even the salicyl group, to antirheumatic efficiency is illustrated by the high efficiency of cinchophen,



and neocinchophen



which do not contain these groups. However, they are chemically related to salicyl, and their structure indicates the presence of the quinoline ring which acts as an antipyretic. Obviously important factors with all the compounds are general solubility and absorbability. The poor solubility and absorbability probably explain the relative inocuousness of neocinchophen. Novaspirin (anhydromethylenecitryl salicylic acid), for instance, is claimed to be non-toxic, etc., but its absorption amounts only to about 17 per cent (median), yet its salicyl content is high, 10 grams being the equivalent of 7.3 grams of sodium salicylate. Finally, the combined use of morphine and quinine, both of which are chemically different from all the drugs thus far mentioned, but which are nevertheless therapeutically efficient in rheumatic fever, indicates the relative unimportance of chemical composition and structure of these therapeutic drugs, and of the specificity of salicyl, in this disease. The speculations on the chemical side of this question have not led to anything definite pertaining to the mechanism of the antirheumatic action.

Salicyl on streptococcic immune bodies

This has been studied by Swift (463, 464). He determined the effects of daily doses of from 0.16 to 0.2 gram per kilo of sodium salicylate gastrically on the development of immunity in rabbits that had received intravenous injections of *Streptococcus viridans* both living and in the form of vaccines, and of washed sheep erythrocytes. As compared with the controls, the salicylized rabbits showed a depression of antibody, agglutinin and hemolysin formation and of complement fixation. The intravenous injection of antigens previously treated in vitro with sodium salicylate showed lower antibody curves than did the rabbits receiving untreated antigen intravenously and salicylate gastrically. The depressant action was, therefore, due partly to some direct action of salicyl on the antigen, and partly per-

haps to decreased rate of absorption of antigen in the salicylized animals. This, however, is no contraindication to the administration of salicyl in the disease. Swift suggests that the beneficial effects of salicyl in rheumatic fever probably can not be attributed to increased production of circulating immune bodies against the infectious agent. On the other hand, the depression of the irritative properties of the etiologic agent suggests a basis for the antiphlogistic action of the drug. However, a difficulty in all this is the uncertain etiology of rheumatic fever. Swift's results with salicyl at least agree with the current opinion that few, if any, drugs or simple chemical substances enhance the formation of immune substances. There are no studies with cinchophen.

Salicyl in experimental arthritis

The response of this condition to salicyl has been tested by severa investigators, but unfortunately the results with both chemical and bacterial arthritis have not suggested the basis of the high efficiency of salicyl and cinchophen in the polyarthritis of rheumatic fever. The selective distribution of salicyl in the joints of rabbits injected with streptococci and in those treated locally with mustard and croton oils has been discussed in the section on distribution; the results with the former were uncritical, and with the latter, negative. The following are experimental results along symptomatic lines.

J. L. Miller (443) observed that, in rabbits, the prophylactic use of salicyl had no value in preventing arthritis after intravenous injection of hemolytic streptococci. Davis (465) produced arthritis in young rabbits with various streptococci and then injected from 0.2 to 0.3 gram sodium salicylate once daily for 2 weeks. The salicylized rabbits died frequently before the controls and Davis concluded that the drug had no prophylactic or therapeutic effect during infection. However, the method of medication used did not conform to that employed clinically. That is, probably saturation of the organism with salicyl is of greater importance than gradual treatment with divided doses as employed by him. The total dosage administered in his animals was large. It is suggested that probably the early death of the drugged rabbits was due to nephritis caused by the salicyl superimposed on any renal injury caused by the infection. Experi-

ments essentially along the same line as Davis' were carried out by Fantus, Simmonds and Moore (466) on rabbits infected with hemolytic streptococci. The dosage of sodium salicylate was 0.5 gram administered in solution and in capsules. The drug was harmless to the controls, but detrimental and fatal to the infected rabbits and the addition of sodium bicarbonate did not alter the result. Acetylsalicylic acid was more toxic than sodium salicylate, and salophen had no influence on, or was unfavorable to, the infected rabbits. Beneficial results in rabbits infected with hemolytic streptococci secured during acute tonsillitis in patients were obtained by Simmonds and Moore (467) on exposure of the rabbits to electric light continuously. All told 22 rabbits were used, and about one-half the joints of the treated rabbits were involved, the lesions being more severe in the untreated controls. The temperature remained unchanged, but there was a greater gain in body weight in the treated rabbits. In another study by Simmonds and Moore (468) on rabbits infected with hemolytic streptococci, the heat factor from exposure to incandescent electric light was considered. The temperature was from 4° to 6°C. above room temperature, amounting to from 33° to 35°C. Six series of rabbits were used. The controls did not die, but seemed to have more marked lesions and gave evidence of greater distress, while large numbers of the treated rabbits died though the lesions seemed to be benefited. The untreated rabbits gained more in body weight. From these results it seems that almost any treatment may be harmful to rabbits with experimental arthritis, and it may be that the hemolytic streptococci produce too severe a disease in this species.

Swift and Boots (469) compared the therapeutic efficiency of sodium salicylate in rabbits infected with the more severe hemolytic, and the less severe non-hemolytic, streptococci. All 4 rabbits infected with the hemolytic streptococci, and of which 2 received sodium salicylate gastrically, died within a few days. All had purulent arthritis, but the organisms were not recovered from the joints. In a larger series of rabbits inoculated with non-hemolytic streptococci, the results were no better as to prevention of death. The rabbits received 0.16 gram per kilo of sodium salicylate daily for 5 days and then 0.08 gram for 2 days, salicyl also being given before the infection, and died sooner than the controls. Latent infections seemed to be lightened up by the salicyl

medication and the cardiac lesions were uninfluenced. Swift and Boots were of the opinion that earlier death in the salicylized rabbits was due to a summation of the bacterial action on the kidney and nephrotoxic action of the salicyl. As to the severity of the arthritis in the treated rabbits, some suggestive results were obtained. About one-half of the abnormal joints of the salicylized rabbits were found mildly inflamed, while all the controls had severe arthritis as though half the joints in the treated rabbits were improved. These are probably the first favorable results with salicyl in experimental arthritis recorded in the literature. However, previous investigators usually employed hemolytic streptococci, which, in the experience of Swift and Boots, usually cause purulent arthritis, such lesions being more nearly comparable to those of osteomyelitis, and different from the arthritis of rheumatic fever. These authors state that, since no clinical benefit is obtained with salicyl in osteomyelitis, none would be expected in rabbits with arthritis from hemolytic streptococci. However, the beneficial results in the arthritis of non-hemolytic streptococci did not agree with the mortality results, since 36 per cent of the treated rabbits died and only 8 per cent of the controls. The higher mortality signified diminished general resistance, a contrast with the apparent beneficial influence on the local infection. The increased mortality under salicyl agreed with the reduction in immune bodies shown by Swift (464). After considering various possibilities, such as a local antibacterial action of the salicyl, a reduction in the reaction of tissues to streptococcic irritation and a strong antiphlogistic action, as in mustard oil chemosis, claimed by some, Swift and Boots concluded against salicyl having exerted a beneficial action because the arthritis was neither completely inhibited nor cured as is that of rheumatic fever. They prefer to leave the question open until milder types of arthritis result from experimental inoculation and the visceral lesions resemble more closely those of patients. What anti-inflammatory action of salicyl there was occurred in rabbits inoculated with streptococci of lowest virulence.

These results suggest lines of attack in the future. Besides choosing organisms of low virulence, it would seem advisable to select a more susceptible species than rabbits for the production of arthritis. According to Hutyra and Marek (470), the acute arthritis of animals is most

common among cattle, cows being more susceptible than steers. In fact, it appears that in this species the changes in joints, endocardium and serosae, the course of infection and the causative factors are nearly identical with those of rheumatic fever in man. In dogs, horses, pigs, goats and sheep, arthritis seems to be rare. Then, the chemical and physical aspects merit consideration. In connection with the greater susceptibility of cows than steers, the possibility suggests itself that the drain on calcium in milk cows favors an increased permeability of endothelial membranes (joints, endocardium, etc.) and greater susceptibility to exudation, swelling, etc., from irritative bacteria or toxins, or both. Also, the seasonal variation in the calcium content of blood, believed to exist by some, might be a factor (when the concentration is reduced) in the seasonal appearance of arthritis in man. It seems that the metabolic aspects, at least calcium and other salt balance, should not be overlooked in determining the etiology and the exudative manifestations of rheumatic fever, even though these may be only contributory causes. Evidence is gradually accumulating to indicate that the physical-chemical relationships of cells and tissues alter, if not determine, their functional state and responses to all sorts of stimuli. This is nicely illustrated by the variety of manifestations in the general group of allergic phenomena among which edema, exudations, disturbances in the circulatory (capillary) and muscular functions figure so prominently. In all these phenomena, cell surface changes, especially alterations in permeability, must be of great fundamental importance. This brings us to the antiphlogistic action of salicyl and cinchophen.

Antiphlogistic action

This pertains to the relief of the inflammatory edema, effusion and pain in the joints all of which are effectively relieved by both salicyl and cinchophen. To what extent antipyresis and analgesia contribute to the relief of the local inflammatory changes in the joints of rheumatic fever is not known. However, in ophthalmia unaccompanied by fever, salicyl has been a favorite remedy for a long time. In the experimental arthritis of rabbits discussed above, the antipyretic action of the drug may be a contributory factor in the relief of the joint changes, hence this experimental condition is not suitable for determining

whether the antirheumatic drugs directly ameliorate the local changes or not, i.e., in the true antiphlogistic sense. The question has been tested with both salicyl and cinchophen in experimental irritative edemas of the conjunctiva, lungs, and head and neck, with favorable, though uncritical, and unfavorable, results.

Mustard oil chemosis. Starkenstein and Wiechowski (230) reported that the mustard oil chemosis of rabbits could be completely prevented by the previous injection of 0.5 gram per kilo of cinchophen subcutaneously 1 hour before. The preventive action was marked and constant. They argued that it could not be due to a fall of body temperature, since there was only a fall of a few degrees in rabbits and dogs. It was also not concerned with a peripheral anesthetic action. Local application of cinchophen gave negative results. The following striking analogies in the actions of cinchophen and calcium appeared to exist; both lowered body temperature, inhibited inflammation, caused emesis sytemically and central paralysis and inhibited glycosuria. The antiphlogistic action was not explained by analgesia, anesthesia, vasoconstriction or a local astringent action, but appeared to be concerned with some fundamental change in the tissues. In a later study, Starkenstein (400) reported that non-toxic doses of cinchophen completely inhibited chemosis of mustard oil in rabbits and of dionine in patients. Lowering of temperature could not explain the inhibition because antipyretics in general were ineffective. However, he found that antipyrine and salicylate, both of which are antipyretics, inhibited the chemosis though more slowly than cinchophen. In cats, chemosis was not prevented by cinchophen and the narcosis from the drug was stronger than in rabbits; quinine and magnesium also inhibited it. Believing that cinchophen acted similarly to calcium, Starkenstein (471) treated chemosis with combinations of the drug with calcium and also with santal oil and found that the preventive action of cinchophen was potentiated by calcium. The action of cinchophen on purine metabolism was also favorably influenced by calcium and it was believed assisted the antiphlogistic action. On the basis of these results, Starkenstein (472) extolled the use of cinchophen in the treatment of spotted fever in which the primary symptom is characterized by vascular changes; of the combination of cinchophen and calcium in inflammations with the idea of preventing the passage

of toxic agents from the circulation into the tissues; of the combination of salicyl and antipyrine in pneumonia, and of cinchophen, in place of salicyl, in rheumatic fever. He (473) suggested that the so-called antiphlogistics, including cinchophen, quinine, calcium, salicyl, serum plasma, milk, methylene blue, fuchsin, iodine, collargol, physiological salt solution, etc., which he tested in chemosis, owed their action to a general protoplasmic or "omnicellular" action in the sense of non-protein therapy and involving an alteration of the entire organism toward toxic conditions. The principle of a diminished vascular permeability did not seem applicable considering the large variety of chemically and pharmacologically different agents acting similarly on chemosis, and the vascular effect did not go parallel with the therapeutic.

The generalizations of this author from the anti-chemotic action of cinchophen and other agents are too sweeping and unsupported by critical evidence. It does not follow that an antiphlogistic action in arthritis, or in systemic infections, would have the same basis as in chemosis. Moreover, critical studies of the mechanism of chemosis and certain other edemas have shown that the inhibitory actions of certain agents are concerned with circulatory depression rather than with the direct actions of drugs on the edematous tissues. It has been shown by Hirschfelder (474) for mustard chemosis and by Tainter and Hanzlik (475) for the edema of paraphenylenediamine that an adequate level of blood pressure (at least 85 mm. of mercury) is necessary in order that the edema changes may be demonstrated. A full discussion of the relationship of various functions to the development of chemosis and certain edemas will be found in a recent paper by Hanzlik (476). The majority of the agents employed by Starkenstein, including cinchophen and salicylate which were used in large doses, are capable of producing circulatory depression and this was not considered or excluded. Unless precautions are employed to maintain an adequate intravascular pressure and circulation through the affected part, inhibitory results, which are obtainable with the antichemotic and antiphlogistic agents discussed above, are apt to be misleading. The same applies to the antichemotic effects of quinine, morphine, antipyrine and salicylate in rabbits reported by Januschke (477) and frequently quoted by Starkenstein and others in support of their

claims; and also to those of cinchophen reported by Dohrn (478). All of these drugs cause general depression and all but morphine depress the circulation. It is interesting to note that the mustard chemosis of cats does not respond to cinchophen, a fact reported by Starkenstein and also by Heubner and Gildemeister (479), who used 0.1 gram per kilo subcutaneously.

From all this, therefore, the antichemotic actions of cinchophen and salicyl are variable and undependable, and, when obtained, they probably occur at the expense of circulatory depression, all other mechanisms that have been suggested amounting to nothing more than mere speculation. Moreover, since any antichemotic action probably depends on circulatory depression and collapse, this does not conform to the occurrence of antiphlogistic action under clinical conditions. Dohrn (478) claimed the discovery of an important principle in antiphlogistic action, namely, that in general the carboxylic acids, which possess antiseptic and antipyretic actions, also act as antiphlogistics though in varying degrees. However, this appears to be only a coincidence and may be waved in view of the more obvious factors pointed out. Some text-book writers and clinicians have attributed the benefits of antichemotic drugs, including cinchophen and salicyl, to central analgesia, and supposed that this basis accounts for the therapeutic results from these agents in rheumatism, laryngitis, bronchitis, and pleurisy, not only as to relief of pain, but also as to actual abbreviation and inhibition of the pathological process. They have further recommended their use in the treatment of a variety of inflammations and infectious diseases. Such unwarranted deductions and generalizations made on the basis of the seriously defective and uncritical experimental results in chemosis discussed above have led to an unjustifiable commercial exploitation of these products for all sorts of conditions, in certain of which these drugs may do more harm than good. Consider, for instance, the possible injurious effects of these drugs on the kidney and heart superimposed on the injuries caused by the disease.

Mustard dermatitis. The importance of circulatory collapse and diminished blood flow through the edematous region in the antagonistic action of cinchophen is illustrated by some recent experiments of Fuerst (480). Fuerst determined the lowest effective concentration of

mustard oil producing dermatitis before and after the administration of cinchophen. The mustard oil was used in the form of small papers soaked in 30, 40, 50, 60 and 70 per cent mustard oil in olive oil and applied to the shaved skin of rabbits. Cinchophen, in very large doses, prevented the dermatitis in 66 per cent of the cases. Simultaneously these rabbits suffered a diminution in rectal and skin temperatures, and there was marked depression, but those rabbits kept warm in an electrically heated box responded with the usual dermatitis, all of which indicates that the prevention, in the successful cases, was due to circulatory and general collapse. Fuerst attributed the inhibition to diminished blood flow in the skin, which, of course, would be expected in collapse. On the other hand, the administration of calcium chloride and hydrochloric acid prevented the dermatitis independently of collapse, and there was no lowering of skin and rectal temperatures. The acid-base equilibrium of the blood was disturbed after both of these agents, and it appeared that a diminution in alkali reserve was responsible for the beneficial result. After cinchophen the carbon dioxide of the blood remained unchanged.

Pulmonary edema of phosgene. E. Lacquer and R. Magnus (481) tried cinchophen and sodium salicylate in cats and rabbits suffering with pulmonary edema from phosgene. They found that doses of from 0.1 to 0.15 gram per kilo of cinchophen dissolved in sodium or ammonium hydroxide, which were not fatal and caused either no or only a delayed and temporary fall of body temperature, caused a greater number of fatalities than occurred among the untreated controls. Sodium salicylate, in single doses of 0.15 gram per kilo or daily doses of 0.25 gram per kilo subcutaneously, had no beneficial influence on, or caused no recoveries among, the phosgenized animals. These investigators, therefore, concluded that these drugs were worthless in this irritative edema. It is true that in these experiments fatal concentrations of phosgene were employed and the pulmonary edema was severe, but under the same conditions doses of 0.167 gram of calcium chloride per kilo possessed some prophylactic value. All this emphasizes that the additive effects of cinchophen and salicyl in experimental pathological conditions are apt to be detrimental, if anything.

Edema of paraphenylenediamine. In this peculiar, specific edema of the head and neck involving the cervical skin, lips, nose, orbits,

tongue, vocal cords and conjunctiva, the antiphlogistic effects of cinchophen, neocinchophen and sodium salicylate have been tested by Hanzlik and Tainter (482) with completely negative results. The blood changes in this edema consist of a marked concentration of corpuscles, hemoglobin and total solids owing to escape of plasma due to increased vascular permeability in the edema regions. The blood changes, which served as an index of permeability changes, and the appearance of the edema grossly during life and at autopsy were employed as the criteria in the study of the effects of the drugs. Sodium salicylate was administered subcutaneously in doses of from 0.15 to 0.2 gram per kilo singly and divided. Cinchophen, dissolved with the aid of sodium hydroxide, was administered gastrically in doses of from 0.1 to 0.4 gram per kilo, and neocinchophen was given as a suspension in water, using doses of from 0.25 to 0.5 gram per kilo. These doses corresponded to the full therapeutic used clinically in rheumatic fever. The drugs were given before the edema was produced; with salicylate this was from 45 minutes to 1 hour, with cinchophen from $1\frac{1}{2}$ to 3 hours and with neocinchophen from 2 to 3 hours before the administration of paraphenylenediamine. At least 4 rabbits were used with each drug, and untreated control animals were observed at the same time.

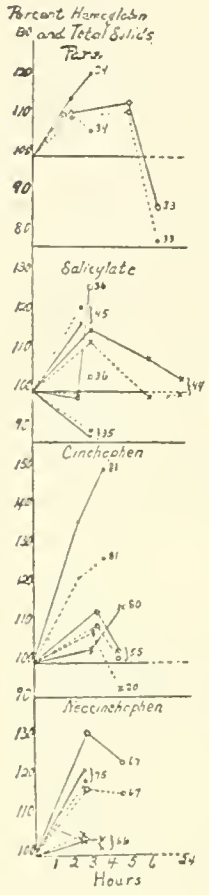


Fig. 10. Sodium salicylate, cinchophen and neocinchophen on the increased blood concentration in the edema of paraphenylenediamine in rabbits. The continuous lines denote changes in hemoglobin, and the broken lines, blood solids.

The results on blood changes presented in figure 10 show that salicylate, cinchophen and neocinchophen did not in any way mitigate or prevent the increase in vascular (capillary) permeability of this edema. The blood concentration was increased as much in the treated as in the control ("para") rabbits; it only decreased shortly preceding, or at time of, death, and this also occurred in the controls, a phenomenon commonly accompanying collapse or death from any cause. As far as gross edema changes were concerned these were aggravated, if anything, by the drugs, especially by cinchophen, which favored the occurrence of peritoneal

and pleural exudates. All three drugs hastened death, which is further evidence against protection, because death in the edema of paraphenylenediamine is mechanical, that is, it is of asphyxial origin due to the swelling of vocal cords, etc. Thus, it is seen that, in this irritative edema occurring without fever, the antiphlogistic effects of salicyl, cinchophen and neocinchophen were not demonstrable, a result in agreement with the negative results of Heubner and Gildemeister in chemosis and of Lacquer and Magnus in pulmonary edema.

It is possible that the inflammatory changes, including the swelling, edema and exudation, in the joints of rheumatic fever are different from the experimental pathological lesions of other regions produced by chemical agents, yet there is no reason to believe that the fundamental changes in the organs and tissues are radically different. It was seen from the experiments of Swift and Boots (470) that about half the joints of arthritis in infected rabbits were somewhat benefited by the administration of sodium salicylate, yet these authors waived the conclusion that salicylate acted beneficially. Therefore, taking all the results together it appears that an antiphlogistic action due to an organotropic influence, experimentally at least, must be denied to salicyl, cinchophen and neocinchophen.

Treatment of rheumatic fever with miscellaneous agents

The fact that the symptoms of rheumatic fever have been successfully relieved, and the disease apparently cured, by a variety of agents chemically and physically different from salicyl and cinchophen is a strong argument against the specificity of these drugs. Successful treatment of a case of rheumatic fever with a vaccine, and one which would not respond to salicyl, was reported by Rosenthal and Widal (483). J. L. Miller and F. B. Lusk (484) obtained prompt recoveries in 29 out of 45 patients receiving from 4 to 75 million typhoid organisms as a vaccine. The pain, redness and swelling disappeared in from 1 to 5 days and usually within from 24 to 48 hours. The endocarditis was benefited as well as the arthritis. These patients showed no improvement after the administration of 1 to 2 grams of sodium salicylate every 4 hours. The remaining patients showed no or only partial improvement; some required re-injection of the vaccine. Comparatively speaking, the results with the vaccine were said to be

quite as good as in typhoid fever itself in which 40 per cent of cases are not benefited. The beneficial response was preceded by the usual marked reaction to vaccines (marked rise in temperature, chill, severe headache, nausea for a few hours and marked dyspnea), but there were no fatalities. Cecil (485) reported 40 per cent successes with typhoid vaccine without the aid of salicyl, but, in the remaining 60 per cent, no relief or recovery occurred until salicyl was given. Recovery from rheumatic fever may occur without medication, but Cecil believes salicyl to be the best of all the remedies and reserves foreign protein, which is a heroic remedy, for cases which fail to respond to salicyl. Scully (486) reported 40 per cent successes with one injection of foreign protein, 52 per cent with 2 injections. Apparent benefits, especially as to cardiac improvement, have been claimed by Menzer (487) with a polyvalent streptococcus vaccine prepared from tonsillitis of rheumatic fever, and by Gregory (488) in 18 children treated with a streptococcus vaccine prepared from the blood of a boy suffering with rheumatic fever. The results, however, were not exclusively due to the vaccines used, since salicyl appeared to have been used some time during the treatment. Proceeding from the notion that the arthritis of rheumatic fever is an allergic manifestation, an idea that has a certain amount of experimental support, Bauer (489) claimed successful desensitization by non-specific agents, such as caseosan, vaccines, peptone, milk, etc. In the treatment of 26 cases of arthritis with intramuscular injections of from 0.5 to 2 cc. of 10 per cent casein, repeated after 4 or 5 days, Roch and Katzenellenbogen (490) obtained most satisfactory therapeutic results in rheumatic fever. They advise the casein as a supplementary measure. According to Kaess (491) any kind of protein in optimal dosage, subcutaneously or intravenously, gives beneficial results in all kinds of arthritis, a preliminary sensitization frequently giving a better result.

The following non-protein colloids are reported to give full therapeutic benefits in rheumatic fever, and frequently when patients are refractory to salicyl medication. The use of colloidal sulphur, 1 to 2 cc. daily for 2 days intravenously, is reported by Massalongo (492) and Viola (493). It caused rapid amelioration of all symptoms and marked sweating. Giroux (494) affirms the efficiency of this agent, but states that it produces violent reactions, and that colloidal gold

intravenously in a dose of 0.25 cc., then 0.5 to 1 cc., is also efficient. Blumenthal (495) advocates colloidal silver intravenously in patients refractory to salicyl, and Martone (496), from 1 to 5 mgm. daily doses of mercuric chloride intravenously.

Widal (497) quotes the successful use of antipyrine 3 to 4 grams daily, and rarely more than 8 grams, by G. Sée, and by Chauffard who used atropine with it conjointly; of sodium benzoate by Senator; of salipyrine and phenacetine by Masius; of asaprol by Dujardin-Beaumetz, and of phenocoll.

Lampronti (498) claims to obtain best results by injecting 8 cc. of 3 per cent antipyrine directly into a joint, 12 cc. being distributed among other joints so that not over 20 cc. are given during 24 hours. The solution consists of 1.5 grams antipyrine in 46 grams of distilled water, and after sterilization by heat, 4 grams of 0.1 per cent epinephrine solution are added. He claims that the pain ceases almost at once, the temperature falls in a few hours, absorption of the edema follows quickly and the local reaction is slight. Melubrin, which is a pyrazolone derivative closely resembling antipyrine, has been used successfully in 1 gram doses 3 to 4 times daily up to 10 grams in rheumatic fever by Hoppe (499), who claims the effects resemble closely those of salicyl. It acts as a general antipyretic.

Whether the use of the different agents that have been mentioned is justified or not, especially the vaccines, proteins and colloids intravenously, the results that have been reported nevertheless indicate that symptomatic benefits, and even cures, in rheumatic fever are obtainable with a variety of unrelated agents. In fact, the symptoms constitute the only criterion by which the efficacy of antirheumatic remedies can be judged, salicyl and cinchophen included. Accordingly, therefore, the so-called specific remedy (salicyl) falls into the same category as the non-specific (diverse agents), which merely indicates that the beneficial result is not peculiar to, or specific for, salicyl. As to the comparative therapeutic efficiency of salicyl and the miscellaneous agents that is another matter, and there are marked differences of opinion on this point.

Non-specificity of salicyl

From the results that have been discussed thus far, it is apparent that the therapeutic responses with salicyl and cinchophen in rheuma-

tic fever are indistinguishable, and, it may be added, also with certain of the miscellaneous agents just discussed. Yet, it is the salicyl group that is alleged to be the specific remedy in this disease. Why cinchophen is not generally included is not clear. The idea of specificity has no better basis than an old clinical impression, unsupported by objective evidence of any kind, and which seems to have been first expressed by Stricker in 1876, who first recognized the merits of salicyl in rheumatic fever. G. Sée (150), who popularized the drug in France in 1877, had the same idea. This idea has been perpetuated down to the present day and is the conception of salicyl action presented in most text books of pharmacology and therapeutics. However, from time to time doubts of its validity have been expressed, and during the past 10 years or so the idea of specificity among investigators and students of the subject has been abandoned. This position has been taken because of the efficacy of other kinds of therapy in rheumatic fever without the aid of salicyl, and of results from direct comparison of salicyl and non-salicyl medications in the same and different patients.

It is interesting to note that Ehrlich (500) accepted the specificity of salicylates in rheumatic fever on the antiseptic basis, although he realized that the etiology of the disease was unknown. The conceptions which he formulated for salicyl, and which were part and parcel of the principles which he followed in his search for specific chemotherapeutic agents, were about as follows: He stated that there were only 4 agents which exerted a specific action in infectious diseases and these were quinine in malaria, mercury in syphilis, salicylic acid in rheumatic fever, and trypan red and arsenic in trypanosomiasis. Out of the 3 principles in chemotherapy that had to be met by drugs, according to him, namely, (*a*) a strong inhibitory or bactericidal action in vitro, (*b*) relative harmlessness to the organism or host, and (*c*) antiseptic action in the organism, salicylate complied only with the second. From this it is difficult to follow the logic of the postulated specificity of salicyl, but on the contrary would seem an argument against it. What held then with respect to the antiseptic actions of salicyl in vitro and in vivo, holds with equal force now, as indeed has been conclusively demonstrated by the results of the researches cited. This is not the place to consider the etiology of

rheumatic fever though it is obviously a paramount factor in any consideration of the specificity of anti-rheumatic drugs. Here it must suffice to cite only the results bearing on the question with respect to salicyl and cinchophen. In order to be convinced of the fact that the etiology of rheumatic fever remains undetermined one needs only to consult the works of the following authors: Widal (497), Rolly (501), Topley and Weir (502), Riesman (503), Boots and Swift (504), Swift (505, 442), Turnbull (506), Vaughn (507), Giroux (494), W. G. MacCallum (508), and Swift, Andrews and Derrick (509). All this, therefore, completely eliminates the etiotropic action of salicyl from the consideration of its specificity, and leaves only the symptomatic relief.

In 1908, Stockman (510) suggested that too much stress must not be laid on the specificity of salicyl because it gives relief, though partial, in conditions other than rheumatic fever; relapses occur even while the patient is taking the drug, and other drugs are nearly as efficient as salicyl. R. Miller (511) objected strongly to the view that salicylates are specific for rheumatic fever, and cited partial and complete relief in other conditions. He also compared the excretion in urine of normal and diseased individuals and found the rate to be about the same. In the excretory study of Hanzlik, Scott and Thoburn (162), no difference in the avidity of rheumatic tissues from the non-rheumatic was demonstrable because the duration of excretion was the same, although quantitatively the excretion in rheumatic fever was about 20 per cent less owing to increased destruction of the drug. In the statistical study of clinical cases by Hanzlik (298), it was seen that sodium salicylate brought about the greatest number of complete responses in rheumatic fever and partial responses in other conditions, but this might have been due to the greater preponderance of rheumatic fever patients treated. A statistical study could not obviously settle the question of specificity, and at best might indicate only some differences as to therapeutic efficiency of the drug. From a study of 212 cases, Zadek (512) concluded that sodium salicylate was not specific because neither salicyl nor anything else would prevent relapses, and the involvement of new joints or of the heart; salicyl reduced the pain and enabled freer movement of the joints while the temperature declined under it, but the same results were obtained

occasionally by physiotherapy. Frenkel (513) believes that the specific results with salicyl in rheumatic fever are based on a misapprehension; the disease is of a remittent or intermittent character, the natural intermission coming on after taking the drug and the attenuation of symptoms is wrongly ascribed to the drug when in fact the drug is not responsible for it. This is probably overstated, but he claims to have observed, in his clinic, patients going through parallel phases of exacerbation and remission whether salicyl was given or not. Swift, Miller and Boots (284) consider rheumatic fever a self-limited disease. From his extensive studies of rheumatic fever and comparisons of the therapeutic efficiency of salicyl, cinchophen and neocinchophen under controlled conditions, Swift (348, 442) is convinced that the drugs that have been considered as specifics are probably only antisymptomatic in their action, an important feature against the specificity being the frequent discovery of persisting infection. However, he believes that the drugs are still valuable for controlling the distressing febrile, arthritic and cardiac symptoms. Ehrström and Wahlberg (458) deny any effect of salicyl on the infectious process and conclude that salicyl is only a symptomatic remedy, and Libarona (514) states that the drug does not differentiate rheumatic fever from other arthritic conditions.

A direct test of the question of specificity of salicyl in rheumatic fever was made by Hanzlik, Scott and Gauchat (441). The method consisted of the comparison of salicyl and non-salicyl medications in patients who were in bed and receiving no other medication. The patients were first treated with non-salicyl medication, and if no relief was obtained within a reasonable period of time, as indicated by the progress of the condition, sodium salicylate up to salicylism in the usual way was administered. That is, the antipyretic and analgesic properties of salicylate were imitated by combinations of other drugs such as morphine or papaverine, and quinine or acetphenetidine, which are chemically different from salicyl, but possess similar antipyretic and analgesic qualities. In this way, the symptoms could be readily combated, and if complete relief ensued without further medication, no specificity for salicyl, either etiologic or symptomatic, could be claimed. The criterion of complete relief was the prompt and permanent relief of all subjective and objective symptoms

of the disease; partial relief was the partial or imperfect relief of the symptoms and the persistence of symptoms unabated was called "no relief." Rather large doses of the combined opiates and antipyretics were used, as this was believed to be correct in principle, for it appeared that the prompt and full therapeutic response to salicyl was due to the marked effects of the massive doses employed. Figure 11 illustrates the comparative antipyretic efficiency of the combined opiate-antipyretic medication and salicyl in the 9 rheumatic fever patients that were observed. This figure may be compared with

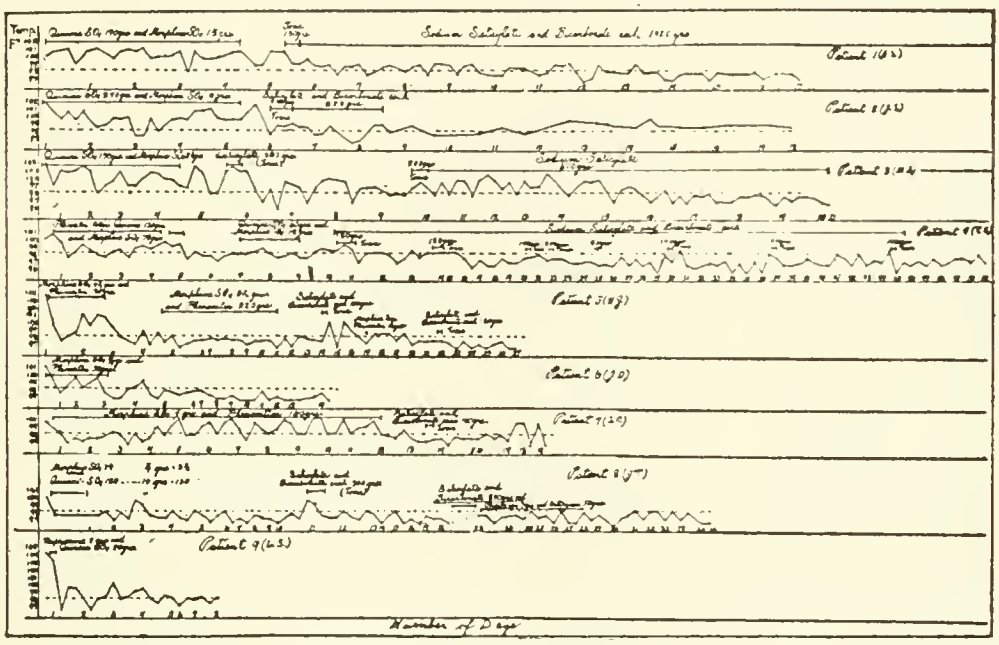


FIG. 11. COMPARATIVE ANTIPYRETIC EFFICIENCY OF NON-SALICYL (OPIATE-ANTIPYRETIC COMBINATIONS) AND SALICYL MEDICATION IN RHEUMATIC FEVER

figure 7, illustrating the results with 8 patients receiving salicyl alone, and which served as a further control for the opiate-antipyretic group.

In brief, prompt though partial relief was obtained in the majority of patients receiving the non-salicyl medication. This was true for both early and late symptoms. In some patients, the symptoms persisted despite salicyl medication, and in at least 4 out of the 17 patients (2 receiving the non-salicyl and 2 salicyl medication) there was a recurrence shortly after treatment with salicyl. In those patients in whom the symptoms persisted, although considerably moderated, after treatment with the non-salicyl antipyretics and

analgesics, salicyl appeared to give more permanent relief. On the other hand, in 2 of the patients the fever persisted in spite of the salicyl medication. In some of the patients, the persisting antipyretic action during salicyl medication, although other symptoms of the disease were present, may have been due to the retention or delayed action of the non-salicyl antipyretic. Four out of the 9 patients receiving non-salicyl medication were not relieved of any of the symptoms until salicyl was administered. Eliminating the elements of time, rest, and natural recovery, it appeared that the relief of later symptoms was brought about more effectively and permanently by salicyl, than by non-salicyl drugs. Although the number of patients was not large, the results, as far as acute symptoms of rheumatic fever are concerned, showed definitely that these can be promptly and effectively relieved by combinations of drugs whose pharmacological actions are similar to, but which are chemically different from, salicyl. It is also possible to obtain permanent relief with combinations of non-salicyl antipyretics and analgesics. Since these results agree with those of several other investigations in which the problem has been approached from other angles, as indicated above, there is therefore a large amount of collective evidence, entirely unopposed, against the idea that the salicylates are specific in rheumatic fever.

Mechanism of the antirheumatic action of salicyl and cinchophen

After all that has been said throughout the text thus far, it remains only to recapitulate briefly the important evidence bearing on, and conclude as to the probable nature of, the mechanism of therapeutic action of salicyl and cinchophen in rheumatic fever. Special attention to the elucidation of the mechanism of salicyl action has been paid only in more recent times, credit, however, being due to the modest efforts of Binz (52, 457) in 1876. The long period of sterility in this direction is to be attributed to the blind patronage of the fallacious postulate of specificity. All the evidence is against the antiseptic action of salicyl under conditions of the body. There is no etiotropic action for the simple reason that the etiological factor is not understood, and for this reason a satisfactory experimental arthritis has not yet been produced to test this proposition directly; and from the fact that antisepsis against an organism, whether bacterium or

parasite, is improbable. An antiphlogistic action through a strictly local organotropic influence is experimentally untenable. The only possibility that exists along this line is an altered (increased) permeability of the vessels with changes in local blood flow, or of articular tissues, or both, which might facilitate the absorption of fluid, toxins, products of inflammation, etc., from the joints. This is not a mere fancy, for an increase in permeability of the kidney to uric acid has been demonstrated with both salicyl and cinchophen, and of living loops of intestine to salts with salicyl, but as far as the joints are concerned it amounts to no more than speculation. Measurements of blood flow through the joints do not exist. Against the idea is clinical evidence of the prompt recovery of the joints without medication. That leaves antipyresis and analgesia, which are definite actions of both salicyl and cinchophen. Both actions occur in a variety of conditions other than rheumatic fever, as will be indicated in the following section. Antipyresis has been demonstrated experimentally and its cause is well understood, though not so the analgesia. Nevertheless, the antipyretic and analgesic actions are general and unquestionable. Hence, it appears that the relief of rheumatic fever by salicyl and cinchophen is mediated through the antipyresis and analgesia produced by these agents, which act rather efficiently, permitting comfort and rest while the joint symptoms disappear spontaneously and gradually. These drugs are only symptomatic remedies, which can be safely administered in very large doses, and represent a fortunate combination of both antipyretic and analgesic qualities which make them more suitable, convenient and desirable than the employment of 2 or more drugs possessing the same actions individually. Their high efficiency and desirability as symptomatic remedies in rheumatic fever may be regarded as outweighing any of the seriousness from the temporary disturbances of renal function, and other side actions.

ACTIONS IN MISCELLANEOUS CLINICAL CONDITIONS

In the following summaries it will be seen that salicyl and cinchophen enjoy an extensive usage in a variety of disease conditions etiologically different. This usage depends on symptomatic relief, that is, of pain, fever or inflammatory swelling. It is probably the

analgesia that is most commonly aimed at. All this helps to confirm the notion that they are symptomatic and not specific remedies, although in certain of the miscellaneous conditions they appear to be less efficient than in rheumatic fever. However, many factors enter into their efficiency, of which dosage is obviously one of the most important. Also, most of the miscellaneous conditions are chronic disorders in which a temporary or partial therapeutic response is the rule whatever the therapy. Therefore, only a moderate degree of therapeutic efficiency from these drugs would be expected.

Arthritis deformans. C. F. Kunze (515) stated that the pain was lessened by sodium salicylate, but G. Sée (150) saw no improvement. Mark (516) could reduce the hypercalcemia of this condition on a calcium-free diet though not to normal, and acid, alkali and sodium salicylate changed the ratio of calcium excretion in urine and feces, but did not alter the slight negative balance on a calcium-poor diet.

Chorea. Successful treatment of this condition has been reported by Fraser (517), but usage of salicyl in this condition varies with opinions of its etiological relationship to rheumatic fever.

Chronic gastro-enteritis. Justi (386) advised 0.5 to 0.6 gram for adults as beneficial in this condition. He regarded the use of sodium salicylate as having a favorable influence on digestion. Beneficial effects were obtained by Wagner (518) in treating gastric fermentation, stomatitis, intestinal decomposition and other similar conditions with salicylic acid.

Coryza. Sick (519) recommended acetylsalicylic acid as a prophylactic in one to two doses of 1 gram each to be taken at the first indication of "cold in the head," or "tickling in the throat." For cough, it was recommended by Ebstein (520). It is extensively used, in fact has become a household remedy, in colds, headaches, etc., in tablets or capsules of 0.3 gram, acting more efficiently as an antipyretic and analgesic here than sodium salicylate.

According to Barbour, Lozinsky and Clements (292), neocinchophen gives effects in colds similar to those of acetylsalicylic acid, but the action is slower and somewhat larger doses are indicated; in tonsillitis 1 to 2 grams.

Diabetes. Ebstein and Müller (521) reported a diminution in the volume of urine and a marked reduction of sugar after doses of 15

grams per day of sodium salicylate. v. Brinken (522) reported a disappearance of glycosuria after four doses of 0.5 gram each of salicylic acid followed by 8 to 9 grams of sodium salicylate. G. Müller-Warnek (523) stated that 9 to 10 grams given daily in three to four single doses caused the glycosuria to disappear so long as the agent was used. No side actions were noticeable, except albuminuria, which disappeared when the agent was stopped. In the majority of patients studied by Fürbringer (524), no beneficial effects on sugar and urine excretion were observed. No beneficial results beyond a diminution in sugar excretion were observed by Kaufmann (525) in the majority of his patients with diabetic coma. The production of the diabetic type of acidosis in animals after salicyl was pointed out in the section on blood.

Encephalitis. Carnot and Blamontier (526) reported prompt benefits in a grave case of epidemic encephalitis of the choreic type after intravenous injection of 4.5 grams sodium salicylate daily up to a total of 22 grams, and in a boy with lethargic encephalitis who received 50 grams in 8 days. The same is claimed by Denechau and Barbary (527) in epidemic encephalitis of a woman who received 0.5 gram sodium salicylate in 4.7 per cent dextrose intravenously and 1 gram intramuscularly during 22 days.

Erythema nodosum. H. Ehrlich (528) claimed benefits in a case from "leukotropin" 10 cc. intravenously, and Richartz (529) from cinchophen in 5 cases.

B. Mendel (279) reported benefits from 1 gram "leukotropin" in 7 cases of eczema.

Gout. Kunze (515) stated that the pain in gout was lessened by salicylate. Combined with colchicin, Cullen (530) also found salicylate to be very beneficial in removing pain in gout.

Cinchophen, however, has had a greater popularity than salicyl in gout. The principal basis of its use here is analgesia. Its use in the treatment of this condition appears to have been introduced by Weintraud in 1911 (221), although in 1908 Nikolaier and Dohrn (28) used it in their studies of uric acid excretion in gout on the fancied etiological connection of this metabolite with the condition. Weintraud (236) used doses of 10 grams; the drug also lowered the temperature in typhoid. He reviewed the literature of the time. Better

results in acute than in chronic gout were reported by Deutsch (531) who treated 25 cases; the drug had to be given with sodium bicarbonate owing to undesirable side actions. Georgiewsky (330) reported benefits with daily doses of 0.5 gram for 10 days, and Zuelzer (532) claimed the drug's action was so good as to be diagnostic. Meidner (533) reviewed the benefits of cinchophen and salicyl in gout and other conditions. Retzlaff (223) claimed best results in gout with 2 to 3 grams daily for 4 to 5 days. Klemperer (450) referred to the use of cinchophen and neocinchophen, claiming that the only objective effect understood was the increased uric acid excretion; however, he used cinchophen extensively in rheumatic fever as an antiphlogistic and analgesic. Friedberg (454) reported 11 cases in which 0.5 gram three times daily stopped the pain, a total dosage of from 8 to 10 grams being used.

Influenza. Acetylsalicylic acid, in small doses of about 0.3 gram, is commonly employed here as an antipyretic and analgesic as in "colds." Neilson (534) claims success with sodium salicylate intravenously in relieving the pain, restlessness and toxemia, but advises against its use in pneumonia. Ammonium salicylate is reported to be "curative" and preventive (535); however without evidence to support this, and probably it acts like any salicyl compound. H. Ehrlich (528) claims benefits in this condition with "leukotropin."

Muscular rheumatism. This was seen to be benefited by G. Sée (150), Schuster (536) and Demieville (537), who injected from 4 to 7 cc. first, and later 10 to 12 cc., of 2 per cent sodium salicylate into the painful areas of large muscles; also a case of rheumatic tetanus by Wunderlich (538).

Neuralgia and sciatica. Acetylsalicylic acid, in small doses, is commonly employed in neuralgia. Marked relief was reported by L. Hoffmann (539) after hourly or two-hourly doses of 0.5 gram each of sodium salicylate for ten to twenty doses. Salophen was reported by Lutze (540) as a good antineuralgic. Success in facial paralysis with salicyl ion medication by electrophoresis is claimed by Fiorini (541). In sciatica, Demieville (537) reported good results after intramuscular injection of hot 2 per cent sodium salicylate solution. Good results with cinchophen have been claimed by Hirschberg (542).

Pleurisy with effusion. Köster (543) recommended 1.5 to 3 grams

of sodium salicylate four times daily as an aid in the absorption of the exudate. Beneficial effects were observed in seventeen out of twenty-seven cases. However, according to Leonhardi-Aster (544) the inflammatory process in the pleura remains unchanged. Leonhardi-Aster also observed such side actions as hemorrhagic pleuritis and hematuria in patients taking salicyl. The use of salicyl in certain serous inflammations is also discussed by E. Smith (545).

Pneumonia. Beneficial therapeutic results were obtained with salicylic acid by Sebring (546); the convalescence was shortened, fever fell gradually, the pulse remained full and not overrapid and no cardiac complications were observed. Benefit with "leukotropin" intravenously has been claimed by H. Ehrlich (528).

Psoriasis. Repeated intravenous injections of sodium salicylate (12 cc. of 20 per cent) were employed by J. F. Smith (547) in 57 cases; symptoms disappeared in 16 per cent, and partial improvement occurred in 32 per cent. On the other hand, Maloney (548), using the same method and drug, obtained only slight benefits in 20 cases, and suggested that salicylic acid externally would be as efficient as the sodium salt intravenously.

Pulmonary gangrene. Beneficial results were reported by Berthold (549).

Scarlet fever. Ramond and Schultz (424) claim good results with 6 grams and upwards of sodium salicylate daily until fever and general symptoms have subsided which usually occurs in 3 days. The medication is resumed on the fifteenth day to prevent complications. Under salicyl the complications are milder and mortality low.

Subacute and chronic rheumatism. No beneficial results with sodium salicylate were observed by G. Sée (150). Roch (550) asserted that acetylsalicylic acid lowers the temperature here about as well as in other febrile conditions. After prolonged medication beneficial results were observed by Schuster (536). After application of salicylic acid (as ointment) to the skin, no beneficial effects were obtained by Bourget (64). In podagra, Kunze (515) reported that swelling of the joints is not influenced by the administration of sodium salicylate. In war wounds of joints, Impallomeni (551) used successfully subcutaneous and intramuscular injections of 1 gram of sodium salicylate in 100 cc. containing 0.01 gram of stovaine.

Beneficial effects from cinchophen in chronic arthritis are denied by Oeller (447) and Richartz (529). Partial benefit is claimed by Brahn (552) with "leukotropin" (a mixture of hexamethyleneamine and cinchophen) 8 to 10 cc. intravenously; also by H. Ehrlich (528) who used 10 cc.

Sympathetic ophthalmia. H. Gifford (553) introduced the use of salicyl in this condition. He observed marked beneficial effects and recoveries with sodium salicylate, acetylsalicylic acid and other salicyl compounds. The usual dosage was 30 grains (about 2 grams) and the mechanism of action suggested was depletion of inflammed tissues by the dilation of capillaries. Others, cited by Whitham (110), as having had experiences similar to those of Gifford are, Welton (554), Bane (555), Campbell (556), deSchweinitz (557), C. Wood (558), Webster (559), Baker (560), Heuse (561), Lindahl (562), Widmark (563) and Lees (268). Moulton (564) advises 1 grain (0.06 gram) sodium salicylate for each pound of body weight to be taken in 10 to 14 hours in panophthalmitis from injury; the salicyl prevents blindness. According to Campbell (565), salicyl is of definite value in certain inflammations of the uveal tract especially in sympathetic ophthalmia. Besides lessening pain and promoting rest and sleep, he thinks the inflammatory process is directly benefited. Very large doses give the best results. Acetylsalicylic acid gives more prompt analgesia, and is also useful after operations on the eye; doses of about 10 grains (0.7 gram) before retiring assure rest. The studies of S. R. Gifford (117) on the distribution of salicyl in experimentally infected and normal eyes were discussed in the section on distribution; there were no significant differences. The antiphlogistic action in ophthalmitis presumably, therefore, does not depend on a selectivity of the salicyl, agreeing in this respect with the effects in other edemas.

Varicose veins. Benefits are claimed by Dunbar (566) and Grzywa (567) in over 30 cases. The veins are injected with 1 cc. of 20 per cent sodium salicylate, and, if no local reaction occurs, up to 40 per cent is injected. The total dose of the drug should not exceed 3 grams. This treatment is claimed to be no worse or better than the surgical, but tends to improve the circulation in the legs. It will be recalled that salicyl softens tissues and dilates the peripheral vessels, but the possibility of sloughing should be kept in mind.

TOXICITY

The symptoms of poisoning by salicyl and cinchophen are similar and resemble those of cinchonism, or salicylism. On account of its close relationship to phenol, salicyl was early suspected of toxicity, but it is the derivatives that have been more responsible for the marked and peculiar side actions and fatalities than sodium salicylate. The literature contains no report of fatalities from cinchophen and its derivatives. The fatal dose varies with the compound or derivative and with the species. For convenience, the fatal and toxic doses of the more important preparations in different species determined or reported by various authors and the methods of administration used, etc., have been summarized in table 2.

Fatal dose

The fatal dose of the salicyl group in different animals has been determined by Laborde (568), Walter (265), Blanchier (97), Oltramare (356), Chanoz and Doyon (569), Houghton (77), Block (160), Waddell (296), Dreser (40) and Barbour and Lozinsky (299). From the summary in table 2, it is seen that there is some variation in the fatal doses of any given compound or derivative depending partly on the method of administration and partly on other factors. Consideration of the details will be omitted. In general, it may be said that the fatal dose of sodium salicylate is from 1 to 1.5 grams per kilo for all species irrespective of the method of administration; and that the fatal doses of methyl salicylate, acetylsalicylic acid and probably also salicyl salicylate (salysal) are less, i.e., anywhere from 50 to 80 per cent less. However, the data on the fatal doses of the derivatives are incomplete, but the toxic doses in human subjects, which have been ascertained on a statistical basis by Hanzlik (298), agree with this tendency. The fatal dose of sodium salicylate in man is not definitely known, but may be somewhat less than, or about, 1 gram per kilo. Allaire (570) reported a fatality from 30 grams, and Caussade and Sharpey (571), in their review, report 10 fatalities with doses from 10 to 30 grams. Assuming about 30 to 60 grams for an adult of 60 to 70 kilos, the median fatal dose of 45 grams (range 35 to 60 grams) of methyl salicylate, in the report of Wetzel and Nourse (273), would

TABLE 2

Toxic and fatal doses of the salicyl and cinchophen groups in different species

(Grams per kilo)*

DRUG	AUTHOR AND REFERENCE NUMBER	MAN†	DOG	RABBIT	OTHER SPECIES
		Salicyl group			
Acetylsalicylic acid	Block (160)		0.2, T.		300, T.; horse
	Dreser (40)				1.4, F.; frog
	Barbour and Lozinsky (299)		0.5–1, F.; gastric		
	Hanzlik (298)	8–11, T.; (R F.)			
	Hanzlik and Presho (21)	7.5, T.			
Amyl salicylate	Chanoz and Doyon (569)		0.5–0.8, F.; vein	0.5–0.8, F.; vein	
Ethyl salicylate	Houghton (77)				1.5, F.; guinea pig
Methyl salicylate	Houghton (77)				0.7, F.; guinea pig
	Hanzlik (298)	8, T. (R.F.)			
	Hanzlik and Presho (24)	8, T.			
	Wetzel and Nourse (273)	10, F.; infant‡			
	Wetzel and Nourse (273)	45, F.; adults§			
	Wetzel and Nourse (273)	15, T.; children**			
Salicyl salicylate	Rocco (161)		1.3–1.4, T.	3–4, T.	
	Hanzlik (298)	5–6, T. (R.F.)			
	Hanzlik and Presho (23)	9, T.			

Sodium salicylate	Blanchier (97)		0.5–1, F.; vein	0.5–1, F.; vein	1,F.; frog
	Waddell (296)			1.6, F.; hypodermic	0.8, F.; cat; hypodermic
					0.6, emetic; cat
					0.9–1.1, convulsive; cat
				1.4–1.6, convulsive	0.65–0.75; rat; convulsive
	Caussade and Sharpey (571)	12–20 F.**			
	Dreser (40)				1.4, F.; frog
					0.95, F.; cat, vein
Strontium salicylate	Hanzlik (298)	9–12, T.; (R.F.)			
	Blankenhorn (584)	8–15, T.; (R.F.)			
Cinchophen group					
Cinchophen	Richartz (529)		0.6, F.		
	Barbour and Lozinsky (299)		1.25, F.; gastric		
			0.5, emetic; gastric		

* Abbreviations in the table have meanings as follows: T, toxic (symptoms of salicylism), F, fatal, and R.F., rheumatic fever; > = more than.

† Doses in this column are total doses the smaller doses, of those represented as ranges are median doses for females, the large doses for males; all others are median doses for males. These doses approach the minimal toxic doses, for in the majority of instances the administration had been pushed to, and then stopped at time of, symptoms of salicylism.

‡ The median of doses (10 to 15 cc.) causing severe toxicity in 4 children up to 9 years reported in the paper of Wetzel and Nourse.

§ The median of doses (30 to 60 cc.) in 3 adults reported in the paper of Wetzel and Nourse.

** Ten cases of fatal poisoning, presumably adults; sex not stated.

TABLE 2—*Continued*

DRUG	AUTHOR AND REFERENCE NUMBER	MAN†	DOG	RABBIT	OTHER SPECIES
Cinchophen group—Continued					
Cinchophen	Hanzlik, et al. (291) Starkenstein (320)	11, T.	1–2, paresis	0.5, delayed death; hypodermic	0.8 convulsive; frog, sodium cinchophen
Ethyl ester of cinchophen	Miller and Boots (295)	11, T.			
Neocinchophen	Barbour and Lozinsky (299)		>50, gastric		
	Hanzlik, et al. (291)	15, T.			
	Barbour, Lozinsky and Clements (292)	10–16; (R. F.)			

indicate that this ester and the sodium salt are about equally toxic.

The fatal doses of cinchophen and its derivatives have been determined only to a limited extent, but, in general, cinchophen would seem to be about as toxic as sodium salicylate, though less than acetylsalicylic acid. Neocinchophen, according to Barbour and Lozinsky (299), would not seem to be fatal in any dosage, for a quantity of 250 grams, or ½ pound, was tolerated gastrically by dogs without ill effects.

Clinical toxic dose

This is also called the full therapeutic dose in rheumatic fever. because the salicyl and cinchophen are administered until gastric and auditory disturbances supervene, i.e., the symptoms of "toxicity," these coinciding with the full therapeutic benefit obtained. In a clinical statistical study of the toxicity of different salicylates in 400 patients, three-fourths of whom suffered with rheumatic fever, the median toxic doses for adult males and females, respectively, were found by Hanzlik (298) to be as follows: 180 and 140 grains (12 and 9.3 grams) of the synthetic sodium salicylate; 200 and 135 grains (13.3 and 9 grams) of the natural sodium salicylate; 120 (8 cc.) minims of the methyl salicylate; 165 and 120 grains (11 and 8 grams) of acetylsalicylic acid; 100 and 83 grains (6.6 and 5.5 grams) of salicyl salicylate. For females the toxic dose was approximately 80 per cent of that for males. The toxic dose of salicyl salicylate was about 50 per cent of that of methyl salicylate and of acetylsalicylic acid about 60 per cent of that of sodium salicylate. The toxic dose of the synthetic sodium salicylate and methyl salicylate for about 68 per cent of individuals of both sexes was between 100 and 200 grains (6.6 and 13.3 grams). The toxic doses of the different salicylates was uninfluenced by age between 16 and 75 years; by racial differences; various disease conditions, and therapeutic response with the sodium salicylate. The median toxic doses of the different salicyl derivatives used by Hanzlik and Presho (see table 2) agree well with the statistical results. Individuals showed idiosyncrasy toward toxic doses of sodium salicylate, but no connection was found between this and such factors as age, sex, race and disease condition. Idiosyncrasy generally varied in the same patient, and was not influenced by previous salicylate

medication. The range and distribution of the toxic doses of sodium salicylate will be seen from the curves in figure 12.

According to Archambault (572), the toxic dose of salicyl in children is higher than would be calculated for the age. In children as well as adults, MacLachlan (573) observed that it requires much smaller doses of salicyl salicylate than of sodium salicylate to produce toxic effects.

The data on cinchophen are not extensive, but the reports of Hanzlik, Scott, Weidenthal and Fetterman (291), Barbour, Lozinsky and Clements (292) and Miller and Boots (295) indicate that the toxic

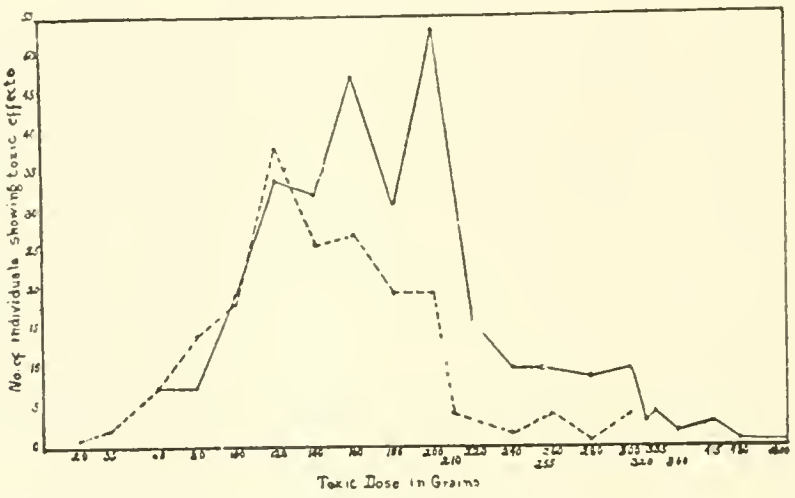


FIG. 12. RANGE AND DISTRIBUTION OF FULL THERAPEUTIC OR "TOXIC" DOSES OF SODIUM SALICYLATE BASED ON CLINICAL STATISTICS

The continuous line represents males; the broken line, females

doses of cinchophen and its ethyl ester are about the same as that of sodium salicylate, i.e., about 11 grams, and of neocinchophen approximately 40 per cent higher, i.e., about 15 grams.

Symptoms in animals

In dogs, Bochefontaine and Chabbert (574) and Laborde (568) observed that an injection of 5 grams of sodium salicylate caused at first a slight acceleration of pulse and respiration and nausea, followed by efforts at vomiting, diarrhea, muscular depression, ataxia, dyspnea, general convulsions, circulatory collapse and death from asphyxia. The same was observed by Blanchier (97) in dogs, and this observer incidentally remarked that if the kidneys are diseased, elimination is

slow, and the accumulated salicyl will give rise to trouble. From experiments on animals, Chirone (575) concluded that the toxic action of salicylates is due to a more or less energetic decomposition of hemoglobin. According to Sée and Lahalle (361), the central nervous system is modified only by toxic doses, when sensation, motion and reflex movements disappear, and death is always preceded by convulsions. Quincke (576) reported the following symptoms; vomiting, dizziness, disturbance of sight, delirium and dyspnea; very large doses slowed the pulse and respiration, and the final picture ended up in collapse and cardiac paralysis. In experiments on rabbits, Charteris and McLennan (577) observed that injections of sodium salicylate caused circular and rotatory motions of the body with paresis and finally paralysis of the hind limbs; progressive prostration with complete muscular paralysis and death. The early symptoms described by Charteris and MacLennan were not observed by Waddell (296), and in rabbits, rats, and cats, the symptoms of intoxication by hypodermic injection of sodium salicylate were early vomiting (within 20 minutes), except those animals that cannot vomit (rodents), reflex excitability and convulsions, terminating in death from asphyxia in about 6 hours after acutely fatal doses; in 18 hours after just fatal doses. Chistoni and Lapresa (141) considered acetylsalicylic acid to be twice as toxic as sodium salicylate in dogs and rabbits, the greater toxicity being attributed to the acetyl radicle. Ethyl salicylate caused nausea and vomiting in guinea pigs, according to Houghton (77).

Toxic doses of cinchophen in frogs, mice, cats, dogs and rabbits were found by Starkenstein and Wiechowski (230) to cause mild convulsions and paresis; coma in dogs and cats. Intravenous injection in rabbits caused central vagus and vasomotor stimulation followed by depression, paralysis, fall of temperature, asphyxia and collapse. Rotter (578) reported that cinchophen was decidedly more toxic in cold blooded animals than in the warm blooded, causing central paralysis in frogs. Starkenstein (320) came to the conclusion that frogs, mice and rabbits were much less susceptible than cats and dogs, the symptoms of toxicity in the latter species being characterized by motor irritation and paralysis. Barbour and Lozinsky (299) found that 20 per cent suspensions (in acacia) of acetylsalicylic acid and cinchophen

were about 10 times more toxic than neocinchophen in rats; neocinchophen was also least toxic to dogs.

Symptoms in man

These are the same for salicyl and cinchophen and are characterized by deafness, ringing and buzzing in the ears, headache, nausea and vomiting, and sometimes diarrhea. With salicyl they were first observed by Stricker (426) and later reported by Peterson (579), Deseille (287), Hall (215), Tuckwell (582) and all subsequent observers. Delirium, mania and hallucinations after salicyl have been reported by Caussade and Sharpey (571), Dahy (580) and Krueg (581). Marshall (583) observed drowsiness with twitching of muscles and suppression of perspiration after 13.3 grams of sodium salicylate. According to Moore (286), nausea can be prevented by the addition of a few drops of chloroform to the salicylate. The toxic dose of strontium salicylate (12 grams) was found by Blankenhorn (584) to be the same as that of sodium salicylate, the symptoms being exactly the same and therapeutic efficiency no better.

Differences in sensitivity to salicylates were reported by Douglass-Hogg in 1877 (585). Richardson (586) cautioned against collapse after salicylic acid. Marked changes in respiration, great increase in pulse rate and collapse were reported by Wecherling (587). Undesirable side actions after 4 grams of sodium salicylate were reported by Lurmann (588). These consisted of a marked fall in temperature with shivering, and of edema of both extremities in a case of arthritis deformans. These effects occurred with repeated administration. Allaire (570) reported similar symptoms together with anuria and lessened secretion of saliva after 50 grams of sodium salicylate. Clayborne reported the presence of absolute scotomata in a patient made toxic with sodium salicylate. Besides the above symptoms, Langmead (590) reported the presence of acetone in the urine and breath of children poisoned with sodium salicylate and acetylsalicylic acid; 2 cases resulted fatally. Recently, Kiess (591) reported vomiting, pain and weakness in 3 subjects ranging from 5 to 22 years after the cutaneous application of 6 per cent salicylic acid in 95 per cent alcohol containing 15 per cent oil of cade. Two children, 5 and 7 years of age, died. These patients were treated for hypertrichosis and scabies and their

bodies were covered with the salicyl preparation. The deaths may have been due to other causes, but the other symptoms were unmistakable. A similar 2 per cent salicyl preparation gave nothing unusual in 200 other children.

An erythema has been frequently met with in the treatment of rheumatism with salicyl. This was reported by Heinlein (592), Erb (593), and Beier (594). Treufel (595) stated that erythema can be avoided by combining salicylates with hyperemia treatment, massage, and baths. Exanthema bullosum after the administration of 4 grams of sodium salicylate is reported by S. Rosenberg (596). There was burning of the skin with edematous swelling of lower eyelids, the face became bluish red and the entire body was covered with bluish red spots; after an additional dose of 7 grams bullae were formed, and a repetition of previous symptoms. Purpura following the administration of salicylate was reported by Ramond (597).

Lees (268) claimed that the administration of sodium bicarbonate with salicylate modified or prevented the appearance of symptoms of intoxication and even convulsions. However, this is not true, for all the symptoms of toxicity occurred in the human subjects of Hanzlik, Scott and Thoburn (339) and of Hanzlik, Scott and Reycraft (163) receiving bicarbonate. Moreover, bicarbonate and salicylate are routinely prescribed together in many quarters and the occurrence of symptoms of toxicity is well known. According to Meara (270), the use of alkalies with salicylates has been dictated more by tradition than by any rationale, and many side actions, such as retinal hemorrhages, are comparatively rare, and may be due to the disease rather than to the drug. A. Lambert's (598) recommendation, in case a particular salicyl derivative produces unfavorable side actions, to try out others in turn until one is found which is favorably tolerated will give only partial success. It will succeed only with respect to a somewhat better taste and gastric tolerance of the less soluble derivatives. However, it does not avoid the symptoms of "toxicity" and possible side actions, for all the members of the salicyl and cinchophen group possess these in common, and, in rheumatic fever, full therapeutic efficiency and toxicity go hand in hand. It is futile to search for non-toxic, yet therapeutically efficient, substitutes or derivatives in this condition. Absence of toxicity means absence of therapeutic efficiency.

The post-mortem changes in fatal poisoning from sodium salicylate have been reported by Quincke (576) to consist of destruction of blood-corpuscles, congestion of most viscera and ecchymoses in serous membranes; cerebral hyperemia by Apolant (599). According to Baldoni (600), therapeutic doses of salicyl salicylate can produce changes in the blood, vessels and spleen. In the spleen a necrosis of the lymphoid elements of the malpighian bodies appeared. Vinci (345) reported nephritis; and in rabbits, Hanzlik and Karsner (341) demonstrated tubular nephritis.

Poisoning from methyl salicylate (oil of gualtheria) has occurred quite frequently, and at least 6 deaths are recorded in the literature. The symptoms are similar to those of poisoning from sodium salicylate, differing only in degree. In McNerthney's (601) case of fatal poisoning with oil of wintergreen in a child 3 years old, the symptoms consisted of nausea, vomiting, epigastric pain, slight opisthotonus, twitching of limbs, dilatation of pupils, rapid pulse, slow and labored respiration, hot and dry skin, and marked thirst. Wetzel and Nourse (273) have recently summarized a number of reports on this poisoning and report a case of their own of fatal poisoning in an infant 21 months old who sallowed 10 cc. of pure oil of wintergreen. Marked ketosis was observed, and major nervous symptoms such as convulsions, chronic or toxic spasms of the extremities and hemiplegia. There were congestion and hemorrhages in viscera, especially multiple subserous hemorrhages of the heart, lungs and meninges, parenchymatous hemorrhages of the brain, lungs and kidneys, and extensive subdural hemorrhages. Other severe poisoning in children from 2 to 9 years have been reported by H. B. Myers (602), H. C. Wood (603), Gallaher (604), Hamilton (605), Stillé (606), and in adults by H. C. Wood (603), Jewett (607), Rosenbloom (271), Rosenbloom and Johnson (608), Legrain and Badonnel (609) and Emenheiser (610). Marked shortness of breath, or air hunger, is emphasized by the majority of reporters; also a semicomatose state, then coma, cyanosis and death. Recoveries among the adults have occurred from 30 cc., and fatalities, in 3 patients, from 30 to 55 cc. Chatin and Guinard (611) found the actions of methyl salicylate and methyl sodium salicylate to be identical. Baum (612) reported an amblyopia of five days' duration after large doses of the essence of wintergreen; Gibson and Felkin (613), excessive miosis and loss of light reflex.

The toxicity of the methyl salicylate is due partly to absorption of the unchanged ester. In the majority of cases of poisoning, the urines have smelled strongly of wintergreen. The excretion of the ester was discussed in the section on excretion. In the patients studied by Hanzlik and Presho (24), delayed and irregular absorption was characteristic. The absorption appeared to be delayed as much as 10 hours, and suggests that chemical antidotes and lavage would be valuable in the treatment of poisoning sometime after swallowing the ester. In Legrain and Badonnel's case there was also a delay, no marked disturbance occurring during 10 hours. Since the cause of death is concerned with circulatory depression and cardiac paralysis as with other salicyl compounds, treatment would be directed at the circulations. All this means that the internal administration of the ester should be done cautiously, if at all, its use being preferably confined to external application.

Since the symptoms of cinchophen and its ethyl ester and neocinchophen are identical with those of salicyl, no further description is necessary. Hanzlik, Scott, Weidenthal and Fetterman (291) found them to be somewhat less frequent and pronounced with neocinchophen than with cinchophen in corresponding doses. The difference is only quantitative, however, and concerned with the poorer absorption and solubility of neocinchophen, for more pronounced and regular toxicity is obtainable with increased dosage. From the results of Miller and Boots (295), about the same holds true of the ethyl ester of cinchophen. As for side actions, the tendency to urticaria was found by Myers, Killian and Chace (293) to be more marked with cinchophen than with salicyl. The more marked gastric irritation with cinchophen than with neocinchophen has been mentioned previously. Besides gastric irritation, Schroeder (614) reported general itching, eruptions, edema, fever, headache, etc., after cinchophen in 17 patients. He advised administration of sodium bicarbonate, calcium as in exudative conditions, and liberal drinking of water with the drug to reduce and prevent these effects. Scully (615) reported an anaphylactoid reaction to cinchophen analogous to that from a foreign protein. Apparently many persons have a peculiar idiosyncrasy toward this drug as they have toward acetylsalicylic acid. Neocinchophen, according to Barbour, Lozinsky and Clements (292), appears to be a

rather inert drug as to side-actions, etc. Oeller (447) claimed that the urine of patients receiving neocinchophen acquires a peculiar characteristic odor which is marked with large doses.

Idiosyncrasy to acetylsalicylic acid

Hypersensitivity to small doses of this derivative is more common than to the other salicyl compounds. It resembles the anaphylactoid phenomena, and is characterized by exudative features, suggesting an increased capillary permeability as the basis of the action, and in which the acetyl group appears to play an important part. The mechanism is not understood, but it may be that the lipoid solubility, and, therefore, permeability, of this compound is better than that of sodium salicylate. It will be recalled that it circulates unchanged in the body, and its toxicity is higher than that of sodium salicylate. From their experiments in vitro indicating some reduced binding of free salicylic acid by serum of asthmatics hypersensitive to acetylsalicylic acid, discussed in the section on absorption, Van Leeuwen and Drzimal (124) claimed that the results indicated a higher concentration of the free drug in the blood exerting a more pronounced action than if it were bound. After all, this does not explain the effects of acetylsalicylic acid, which exists in the circulation as the sodium salt; nor is it likely that the binding of added sodium salicylate would be the same as of salicylic acid.

The following symptoms have been reported: Constriction and edema in the throat with dysphagia and salivation by Otto (616); congestion in the nose, swelling of eyelids, edema of pharynx and salivation by Hirschberg (617), Gilbert (618), and Morgan (619). In Morgan's case, the diet contained fish and other preserved foods of animal origin, so that the urticaria and angioneurotic edema could be explained by this factor. Congestion and edema of the pharynx with salivation and symptoms of choking with dysphagia after 5 to 10 grain (0.3 to 0.6 gram) doses have been reported by Macht (620). Edema of the eyelids and other symptoms of poisoning have been reported by Graham (621), Buhlig (622), after 10 grains (0.6 gram), Hansen (623), and Hearne (624). Kitchin (625) reported the case of a medical student who responded to 0.3 gram of acetylsalicylic acid at the end of 2 hours with severe angioneurotic swelling of the face

and arms, and to second, third and fourth doses with large urticarial wheals on the trunk and limbs and marked itching lasting 36 hours. After 1 gram, Sucin (626) observed swelling of the upper lip, the swelling soon extended to the entire head and was not relieved by cold applications; deglutition was painful and the pulse and respiration were increased. There seems to have been an unusual delay in the appearance of these symptoms. Berlioz (627) is the only one to claim that undesirable side actions are less common with acetylsalicylic acid than with sodium salicylate.

The death of a fetus as the result of the administration of salipyrin has been reported by Teilmann (628).

Proceeding on the assumption that the hypersensitivity to acetylsalicylic acid in their case was analogous to protein anaphylaxis, Widal and Vallery-Radot (629) claim desensitization of a patient, who had reactions to the drug 9 years, by the administration of the drug in increasing doses of from 0.005 to 0.03 gram at 1 hour intervals repeated 13 times during a period of 6 weeks. Labbé and Haguenau (630) proceeded along somewhat the same line, but claimed a kind of cross sensitization, perhaps from dietary articles. They desensitized by giving a small dose of the drug 1 hour before the full dose; the desensitization was temporary. However, the fundamental reasoning here is fallacious, for the peculiar reactions to acetylsalicylic acid occur in previously untreated (unsensitized) individuals, and, therefore, the condition does not correspond to protein sensitization. There would seem to be no doubt that, in such individuals, there is a preexisting altered general functional state, probably in virtue of physical-chemical conditions in the cells and their media. It is peculiarly true that hypersensitive individuals give altered responses to a variety of agents physically and chemically different, and, therefore, there is nothing specific about it. The various aspects of the allergic state cannot be considered here, but a summary of the probable basis has been considered in a paper by Hanzlik (631).

Acetylsalicylic seems to partake of a narcotic action to some extent, as it appears to promote sleep in some individuals. Macht (632) reported habituation in an individual who consumed from 25 to 60 grains (1.6 to 4 grams) daily during 2 years for relief in periostitis and osteitis. The habit grew from taking 5 grains (0.33 gram) for

relief of these conditions. Constipation, digestive disturbances and low blood pressure were present.

"Natural" and synthetic salicylates

Until 1911 to 1913, there was a belief among clinicians, practitioners and toxicologists that the salicylates made from some natural oil (as the oil of sweet birch) were less toxic than those prepared synthetically. This idea has been particularly fostered by interested manufacturers. Among the first to offer experimental evidence that the synthetic sodium salicylate might possess greater toxicity were Charteris and MacLennan (577). From two experiments on rabbits, they concluded that "natural" sodium salicylate was less fatal than the synthetic. Later, Charteris (633) claimed to have established, in experiments on animals, that the greater toxicity of the synthetic salt was due to certain impurities (either accidentally or carelessly obtained during manufacture) such as cresotinic acids, particularly the paracresotinic acid. Dunstan and Bloch (634) found ortho-, meta- and paracresotinic acids in specimens of synthetic salicylate and believed that this would explain the reported toxicities.

Stokvis (635) claimed without convincing proof that the natural acid was less toxic than the synthetic because of its greater osmotic properties, this favoring elimination. Many clinicians of Charteris' time were of the same opinion; however, not a single iota of evidence was offered by any of them that would definitely prove the greater toxicity of the synthetic salicylate. A review of nearly all of the clinical papers on this phase of the question was made by Eggleston (636), who showed there was no definite or serious clinical or experimental evidence one way or the other.

In 1890, Stockman (637) became suspicious of Charteris' experimental evidence and the claims of the older clinicians. He objected to the statement that the toxic impurities in the artificial salicylate was cresotinic acid, because Buss (85) and Demme (638) had used it in place of sodium salicylate in treating children, and without observing any untoward symptoms. May (461) stated that from 90 to 120 grains (6 to 8 grams) of *p*-cresotinic acid had been administered without producing toxic symptoms; and, from experiments conducted by himself, May concluded that the cresotinic acids resembled salicylic

acid as antifermentatives, bactericides, antipyretics, and they relieved rheumatic pain. The toxic effects in animals were about the same, although the ortho acid was more depressing to the circulation. This showed that if the synthetic salicylate contained an impurity no more toxic than the salicylate itself, the greater toxicity could not be attributed to the impurity. The experiments of Charteris were repeated and extended by Waddell (296). Waddell could not confirm some of Charteris' results, and showed definitely on cats, rats and rabbits that there were no demonstrable differences between the toxicities of the "natural" and synthetic sodium salicylates and the sodium paracresotinate. Mixtures of the paracresotinate and the different salicylates exhibited no greater toxicity than the components. The fatal and toxic doses obtained by Waddell have been already referred to, and these were practically the same for the different salicylates and the paracresotinate. Stockman (460) showed, in observations on patients, that the actions of sodium salts of the ortho-, meta- and paracresotinic acids did not differ from those of sodium salicylate.

Later clinical evidence supported these experimental observations on animals and patients. This was obtained from several clinicians in different parts of this country, who were unaware of the character of the salicylate, i.e., "natural" or synthetic, submitted to them for use in treatment of patients ill with rheumatic fever and otherwise. The results were summarized by A. W. Hewlett (639), who could find practically no difference in the toxic symptoms, size of dose or therapeutic responses with the salicylates of "natural" and synthetic origin. All of the reports were in uniform agreement. As to the validity of the claims for the existence of impurities in the "synthetic" salicylates from the chemical standpoint, the paper by Hilpert (16) may be consulted. This emphasizes the fact that salicylates of modern manufacture are practically free from impurities, and these include those of so-called "inferior" as well as "superior" grades. In fact, the "natural" salicylate may contain more impurity than the synthetic. It must be concluded from all this that there are no demonstrable differences in the toxicities and therapeutic efficiencies of the "natural" and synthetic salicylates.

METHODS OF ADMINISTRATION

Oral

This is the most common method used for administering salicyl and cinchophen and is efficient because nearly all the preparations are readily absorbed; the insoluble and unabsorbable compounds are rendered soluble and absorbable partly as such and partly after decomposition. Neocinchophen is so poorly soluble that it can be given only by mouth. There is no good reason why any other method should be generally adopted. Other methods do not avoid the emetic action, since this is central. Nausea from gastric irritation can be reduced by the administration of bicarbonate together with sodium salicylate or cinchophen. According to Weintraud (221), administration of bicarbonate together with cinchophen is indicated to prevent precipitation of urates in the urine. The salicyl derivatives are apt to cause quite as much gastric disturbance as ordinary salicylate. Neocinchophen appears to give the least disturbance, but its expense and the larger doses necessary for therapeutic effects outweigh the small advantages.

For headaches, colds, etc., single doses of from 0.5 to 1 gram of sodium salicylate or cinchophen are usually administered; 0.3 gram of acetylsalicylic acid, and about 1.5 grams of neocinchophen. In rheumatic fever, the intensive method is used and consists of administering 1 gram (or about 0.15 to 0.2 gram per kilo) of any of the members together with an equal quantity of bicarbonate every hour until symptoms of "toxicity" (salicylism) supervene. Then the administration may be stopped, and in many instances no further administration is necessary. Some prefer to continue the administration, using 1 gram 2 to 3 times daily for several days whether symptoms are present or not. Recurrence of the fever or other symptoms is always an indication for further administration. Salicylic acid should not be employed for internal administration on account of the marked gastric irritation. Lecoq (642) ascertained that as little as 0.69 gram of salicylic acid was not tolerated by infants and adults unless neutralized with bicarbonate.

Rectal

This method has been advocated by Heyn (89) after experiences with 122 cases. The following advantages are claimed: (*a*) ease of administration; (*b*) ready absorption; (*c*) minimum of untoward effects; (*d*) easy removal of excess by enema; (*e*) results are more prompt than by other methods. The first adult dose consists of 8 to 10 grams of sodium salicylate dissolved in 120 to 180 cc. of plain or starch water containing 1 to 1.5 cc. of tincture of opium. This is repeated in 12 hours if symptoms of salicylism do not appear. The largest dose given was 24 grams. This would seem to have some advantages for children and for avoiding the taste and gastric irritation, but not toxicity. Irving (640) uses from 1 to 3 grains (0.06 to 0.2 gram) per pound of body weight, in starch paste or mucilage of acacia, once or twice daily in children to avoid gastric irritation. The latter is also testified to by Yagüe (641). The rectal method would be useful in case of anatomical defects of the upper alimentary tract.

Intravenous

F. Mendel (643, 644) recommended the following solution in rheumatic fever; sodium salicylate 8 grams and caffein sodium salicylate 2.5 grams dissolved in 50 cc. of water. One-fourth of this solution (equivalent to 2 grams of sodium salicylate) is injected as a single dose in an adult, and this is repeated every twelve hours for three days or as often as necessary. The effects are prompt. Other advocates of intravenous salicyl therapy in rheumatic fever and other rheumatic affections are: Lesné (645), who injects a 50 per cent solution of sodium salicylate using 0.25 gram for each year of the child's age; Lutembacher (646), who employs a 3.3 per cent solution and 3 grams of the salicylate twice daily; Gilbert, Coury and Benard (647), who inject from 0.25 to 2 grams; Matta (648), who uses 5 to 10 grams; Cernadas (649), who gives daily injections of 6 cc. of a mixture of 5 grams sodium salicylate and 0.25 gram caffeine in 25 cc. of water, and Carnot and Blamontier (526), who use from 4 to 6 grams daily and claim 1 to 3 grams sodium salicylate prevent involvement of the endocardium. The various advantages claimed by these advocates of the intravenous over other methods of administration, namely, the avoidance of gastric disturb-

ance, emesis, and side actions in general, the prevention of cardiac involvement, and more rapid absorption and action are unsupported by a single iota of evidence. The various evidences that have been cited throughout disprove these claims. Absorption is rapid by mouth, the drug is not antiseptic and does not beneficially influence the endocarditis; the emesis is central and gastric irritation can be eliminated by the use of bicarbonate. There is, therefore, no good excuse for giving any of these drugs intravenously. In fact, when administered in that way they can cause considerable harm to the heart and other important organs, resulting in collapse. The object of the caffeine in some of the solutions is to correct or prevent the collapse action. Undesirable physical and chemical changes in the blood, and symptoms, in dogs injected intravenously with sodium salicylate have been demonstrated by Hanzlik, De Eds and Tainter (650); and a diminished permeability of the liver of dogs to rose bengal by Hanzlik and De Eds (651).

The same applies to the use of cinchophen intravenously. This has been used by B. Mendel (279) in the form of "leukotropin," a mixture of hexamethyleneamine and cinchophen; and in the form of "atophanyl," a mixture of equal parts of cinchophen and sodium salicylate containing procaine, by Sundermann (652) and Wessel (653), but is obviously polypharmacal and superfluous, if not dangerous.

Miscellaneous

Hypodermic administration of sodium salicylate has been advocated by Behr (654) and Seibert (655), but strongly opposed by Brugsch (656) on the grounds that it is painful and gives no better, if as good, results as oral administration. No side actions occurred until about 5 grams of the drug were injected. The method is, of course, effective in animals. Intra-articular injection of sodium salicylate has been practiced by Santini (657), but is painful and the method is obviously limited by the inaccessability of many joints. The cutaneous method is employed with salicylic acid and methyl salicylate in the form of an ointment, in oil or as liniment for local effects. For eczema and certain other dermatoses a plaster containing salicylic acid has been recommended by H. S. Klotz (658). The method is useless for securing systemic effects in the treatment of rheumatic fever because the absorption is irregular and inefficient.

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THE SIGNIFICANCE OF THE PHYSICAL CONSTITUTION IN MENTAL DISEASE

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EARLY STUDIES OF BODY TYPES

The question of a connection between external appearance and mental functions has been an ancient preoccupation of the human mind. In following the historical development of this subject, one finds that there enters nearly always into the discussion the preconceived intuitive idea of an essential unity between the morphological

and psychic aspect of the individual. This varies only more or less formally with the development of philosophical ideas concerning the relations of mind and body. It thus happens that until comparatively recent times whenever we look for precise definitions of supposed body types we find psychobiological elements, like physiognomy, general attitude and posture entering into the description, and when we examine descriptions of personality or temperament types we find admixtures of signs taken from humoral pathology, physiology and morphology.[1]

The Hindus (53) (88) distinguished three types of man, called symbolically the hare, the bull, and the horse, and three types of woman, the deer, the mare, and the elephant, supposedly characterized by special qualities of body outline, size of genitals, hair, voice, emotional tendencies, etc. The literature on the doctrine of temperaments, based on Galen's theory of Krasis,[2] contains descriptions with a similar mixture of morphological, physiological, and psychological elements, which makes it often difficult, if not impossible, to determine what the various authors meant by their descriptions of temperaments. This holds true also for the nineteenth century medical literature concerning temperaments. The "classical constitutions," the lymphatic, the sanguine, bilious, etc., were still taught in the course on the practice of medicine by Sir Grainger Stewart at Edinburgh in 1890 (58). An extreme and ludicrous example is the distinction of the hemorrhoid and melancholic temperament by Stahl (81), the forerunner of Lavoisier. It is not astonishing, therefore, that the knowledge of morphological body types made very slow progress. The history of racial anthropology affords an analogy where Linnaeus, the first scientist to give man a place in the "natural system," gave a description of four races, Europaeus albus, Americanus rubescens, Asiaticus fuscus and

[1] Compare the derivation of such words as hypochondriacal, phlegmatic, etc., and the traditional body types of dramatic characters such as Henry Percy (Hotspur) and Falstaff in Shakespeare's "Henry IV," Hamlet, the rejuvenated Faust and Mephisto. In order to realize how closely body type and character are interwoven in the eyes of the spectator, one has only to attempt to visualize Hamlet looking like Falstaff, or Falstaff having the physique of Mephisto.

[2] The Greek word κρᾶσις (mixture of body fluids) = temperies, the root of the word temperament.

Africanus niger in which the main characteristics include skin color, body shape, posture and temperament (Scheidt, p. 5 (75)).

BEGINNING OF MEDICAL ANTHROPOMETRIC STUDIES

In medical literature the studies of morphological-physiological types and temperaments, peculiarly intermingled as they were, were from the beginning of scientific medicine influenced by the important consideration of disposition to disease. Hippocrates knew a habitus phthisicus and apoplecticus. Galen taught that the human constitution (κατασκευή), in the Galenic and post-Galenic writings often used synonymously with habitus (ἕξις), nature (φύσις) and disposition (διάθεσις), comes through a disease process (πάθος) to a state of disease (νόσος) (Günther, p. 2 (28)). It was not until 1860 that Wunderlich, who himself distinguished more physiologically an irritable, strong and limp constitution (Wuth, p. 5 (93)), spoke of a promising program for exact analysis and measurement of the physical constitution (Günther, p. 3 (28)). The rise of cellular pathology on the one hand, and bacteriology on the other, prevented for a long time the realization of this program.

The Italian de Giovanni (24) seems to have been the first physician who attempted on a large scale the investigation of physical habitus in relation to disease. He fell into the extreme of trying to prove that "the cause of the special morbidity of the organism resides in its special morphology" (p. 66). His methods, however, were imperfect, his conclusions very dubious. The historical antagonism between morphological constitutional research and bacteriology is well illustrated in his book, as when he speaks of his reticence toward "the parasitic theories" (p. 96–97) and claims that in the human body the diplococcus of the saliva can be transformed into the pneumococcus and meningococcus (p. 96). Nevertheless, his researches were both stimulating and suggestive and he became the founder of a productive school of clinical anthropometrists (Viola, etc.). De Giovanni described four morphological types, or "combinations," as he calls them, mainly on the basis of the development of body cavities. These "combinations" (to be discussed later) are, as Bauer (5) states, the prototypes of the body forms described by Kretschmer (44), who has attempted to correlate them with the incidence of definite forms of mental disease.

ANTHROPOMETRIC STUDIES IN PSYCHIATRY

In psychiatry, just as in general medicine, consideration of the physical constitution is bound up for a long time with the post-Galenic descriptions of "temperaments" in what we now know to be a strange mixture of fancies and facts. It seems interesting that in the very beginning of scientific psychiatry Esquirol (17) compiled statistics regarding the external appearance of his patients. He made the following table (p. 157):

Habitudes extérieurs du corps	embonpoint médiocre	122
	maigreur	60
	obésité	6
Taille[3]	élevée	102
	petite	19

In the same chapter Esquirol states that generally those patients who are strong and robust (sanguine temperament) tend more to an acute course of the psychosis with more definite crises, whereas others (with a lymphatic temperament) tend to a more chronic and dementing course of the disease.[4] This passage where Esquirol mentions the physical constitution of his mental patients is the more remarkable because it is the only one where Esquirol, whose nosological classification is otherwise purely symptomatic–scholastic, seems to anticipate in a way the modern distinction of affective (manic-depressive) reaction types with acute, attack-like course and schizophrenic reaction types with more chronic and often deteriorating development.[5]

The modern scientific morphological studies in general medicine, undertaken with the view of finding correlations with definite systemic diseases (de Giovanni) were suppressed by the sensational success of

[3] La taille (French) = body height = stature (anthropological terminology).

[4] Compare also Pritchard (70). "It has been observed by M. Esquirol, that when persons of the lymphatic or phlegmatic temperament, or those who have a pale exanguious constitution, fall into mania or monomania, their disorder is more liable than that which occurs in other constitutions to pass into dementia or incoherence."

[5] It may be noted as a digression that Esquirol also knew the occurrence of cyanosis in hands and feet of schizophrenic patients. In the good description of a case under the diagnostic heading of "melancholia," which leaves no doubt that the case is one of what we would now call schizophrenia, he says: "Ses mains sont souvent violettes ainsi que ses pieds."

cellular pathology and bacteriology. They were revived only with the rise of modern endocrinology. This renewed interest in morphology was based on the observation that, due to certain endocrine disorders, individuals show morphological changes and that these individuals, as for example in cretinism and acromegaly, tend to lose their racial and family resemblance, but show a morphological similarity among each other (Wuth (93), p. 1–2). These endocrinological studies were fruitful in certain definitely pathological cases. In regard to morphological (and psychological) types they have yielded very little that may be regarded as definitely established.

The investigation of the physical constitution in psychiatry was deflected in a different direction. Influenced by Gall, though opposed to his doctrines, Morel (61) propounded his theory of degeneration which has influenced psychiatric thought profoundly until the present day. Physical signs of degeneration were the basis of Morel's ideas; he found it necessary in order to understand degeneration to study the "natural history of man." His knowledge of physical signs of degeneration, however, was scanty. He laid particular stress on the variations in the human ear, of which he described one form, the so-called Morel ear. On this subject sprang up a whole special literature in psychiatry. In his later work, "Traité des maladies mentales," Paris, 1860, where he stresses again and again bodily degeneration as the basis of hereditary mental disease, he devoted only five of 860 pages to the description of physical signs of degeneration. The reticence of Morel is in contrast to the enormous expansion of the doctrine of morphological degeneration signs by Lombroso, who studied morphological anomalies primarily from the point of view of criminology. Both Lombroso's anthropological and statistical methods were uncritical. Many of his results were disproved when more exact methods were employed. Little today remains valid of Lombroso's life work, and whatever is left of the physical signs of degeneration, the stigmata hereditatis as Morel called them, belongs to the sphere of constitutional variations and diseases. Theoretically these physical signs of degeneration, as they are wrongly called, are of great interest. Their study with regard to etiology and correlation with other morphological signs is by no means completed. Figure 1 shows an example of a morphological characteristic which has been regarded as

environmentally determined, but which is really constitutional, namely the atrophy of the fifth toe in man. Schultz (79) describes a morphological variation which might easily be mistaken for a constitutional racial stigma but which he proved to be environmentally deter-

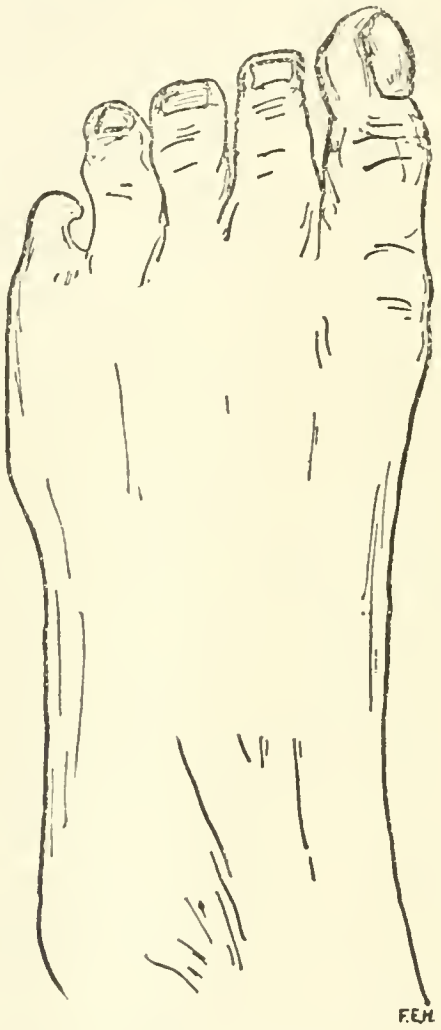

FIG. 1. FOOT OF AN EGYPTIAN MUMMY OF THE XI DYNASTY—ABOUT 1300 B.C.—CENTURIES BEFORE BOOTS APPEARED IN EGYPT

The last phalanx is diminutive, and its joint is ankylosed. From photo by Dr. George Sobhy, Cairo, in Journal of Heredity, 1917.

mined, namely the peculiar position of the fifth finger of the Rama Indian (see fig. 2). Lombroso's interpretation of "physical signs of degeneration," namely, his theory of anatomical-biological regression in born criminals to the morphological status of lower races, prehistoric man and animals (Lombroso's follower, Kurella, spoke of

primatoid skull forms) had to be largely given up. Sommer (quoted by Fischer (19)), who to some extent has recognized Lombroso's teachings, has pointed out that Lombroso has confused two separate problems: first, the problem of whether the delinquente nato really exists; secondly, if he does exist whether this abnormality is characterized by significant morphological features. Just as Lombroso's methods were challenged there was also a strong criticism of the uncertainty in the definition of his object of study, namely what really constituted a born criminal. There is a certain analogy between the formulation of this

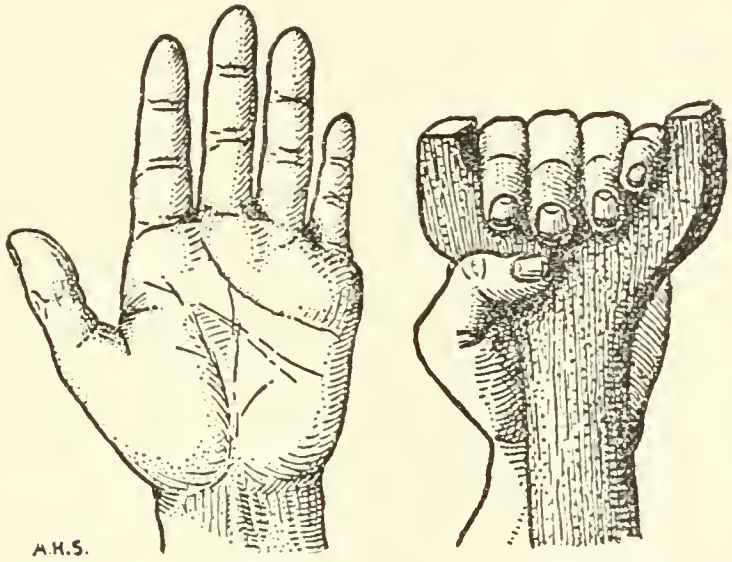

FIG. 2. SKETCH OF HAND OF A RAMA INDIAN AND OF THE MANNER IN WHICH THE HANDLE OF A NICARAGUAN PADDLE IS GRIPPED

Note the position of the little finger. This drawing shows a morphological variation easily mistaken for a constitutional racial stigma.—After Schultz (79).

problem and the problem which at the end of the 19th century confronted physical anthropologists who used exact anthropometric measurements in psychiatry. Having successfully studied and differentiated ethnic groups with modern methods, anthropologists attempted to distinguish with anthropometric methods the "insane" from the "normal" individuals. In an excellent paper, which from the point of view of anthropometric methods is in many ways as useful today as it was then, Hrdlička (35) formulated a program: "The new program is, after being able to define the human normal, to separate the abnormal from the normal, and to classify, if possible, the ab-

normal. And it is here, in this its latest application, where anthropometry encounters the domain of medicine. As an aid in determining the abnormal in man, it serves both the general student of mankind and the clinician, and they really join hands at this point, the anthropologist and the physician" (p. 523). It is, however, exceedingly difficult in psychopathology to make a generic distinction between abnormal and normal. With the closer dynamic study of psychopathological conditions it became more and more apparent and may be regarded as well established today that elements of certain psychopathological reactions reach far into the sphere of psychopathic personalities (that is to say, of people who though constitutionally slightly abnormal may not suffer from a circumscribed mental disease) and even far into the group of normal people. This is true also for the two large and most common psychopathological reaction types, the manic-depressive and the schizophrenic. It is, therefore, not astonishing that generally valid significant morphological correlations with "normals" and "abnormals"[6] were not found, and that on the other hand, with the clarifying of our psychiatric diagnostic conceptions, contrasts between two mental diseases offer a better object of study for anthropological correlations.

Modern heredity studies in psychiatry have gone through an analogous development. The pioneer researches of Koller and Diem have shown that there are no very significant differences as regards the quantitative incidence of mental disease in the ancestry of "normal" and "abnormal" individuals. The tendency of modern heredity studies in psychiatry has been more and more to draw on all even slightly abnormal relatives and finally on all members of the family (see Kahn, p. 48 (38)). Despite the progress of modern heredobiological research in psychiatry, the knowledge of even a complete family history does not give a clue to the pathogenetic factors of psychoses. For this study the modern dynamic and analytic point of view proved more fruitful, and, inasmuch as it emphasizes changeable

[6] The impasse which is easily reached by making the distinction "normal" and "abnormal"—that is to say a "monotype standard"—the starting point of investigations, is of course not confined to psychiatry alone. The early X-ray studies of the stomach where all sorts of atonic conditions were described on the basis of a preconceived normal one-type standard are an example. This subject is well discussed by Mills (59).

psychogenic and environmental factors, more practically and therapeutically useful.

HEREDITY AND CONSTITUTION

However great the advances in the knowledge of exogenic etiological factors of disease have been in the various stages of medical progress, the age-old and unavoidable experience both in general medicine and psychiatry, that different individuals react differently to similar kinds of exposure, has always prevented the suppression of the recognition of hereditary factors of disease, from the "res naturales" of the old physicians to the "constitutional factors" of modern pathologists. The term constitution is used by different pathologists in a very different and arbitrary way. Without going into detail in the examination of these different definitions, it seems correct to say, that most of these formulations are more logically precise than practically directly applicable according to our present state of knowledge. It is not yet possible in human pathology always to distinguish with exactness between hereditary (often used synonymously with unchangeable) and acquired signs. We are forced to assume that the Mendelian laws of heredity apply also to anthropology in the widest sense (Eugen Fischer (18), p. 136). In fact it has been claimed that the only binding proof of the hereditary character of any sign, is the knowledge of its mode of transmission according to the Mendelian laws (Fischer, l. c., p. 137). In the human organism, however, the delimitation of "hereditary units" is infinitely more complicated than in plants. Borchardt (9) comes to the conclusion that it is not possible to divide constitutional anomalies into hereditary and acquired. The concept of constitution cannot have a heredo-biological foundation alone. He therefore also disagrees with Tandler's distinction between constitution and condition. If the difficulty of distinguishing between hereditary and acquired traits be true in the sphere of human morphology it is self-evident in the sphere of psychobiological integration. The formulation of constitution as disposition to disease, as a measure for the resistance of the organism toward pathogenic influences (Martius, Strümpell) is too narrow, since disposition to disease is only one correlative sign or a correlative combination of signs among many others (Lubosch (52), p. 108). To confine constitution to morphology alone

TABLE 1

Classifications of body types

	1	2	3	3a	4
Hippocrates		Habitus apoplecticus	Habitus phthisicus		
Walker (1852)	Locomotive beauty (Diana)	Nutritive beauty (Venus)		Mental beauty (Minerva)	
Carus (1853)	Athletic, lascivious, plethoric arterial constitutions (bones and muscles strongly developed)	Phlegmatic, bocotic, plethoric venous, choleric, apathic constitutions (region of digestive organs prominent)	Asthenic, sensory, pneumatic, chlorotic, phthisic, lymphatic constitutions (narrow chest, long body, skeleton and muscles poorly developed)	Cerebral constitution (delicate)	Sterile, atrophic constitutions (general and special sex characteristics little developed)
Rokitansky-Beneke	Normal type; disposition to unconstitutional diseases only	Types with disposition to carcinoma; hyperplastic	Types with disposition to tuberculosis; hypoplastic		
Kundrat					Primary vegetation disorders (hypoplasias, etc.)
De Giovanni	Second combination; plethoric	Third combination	First combination; phthisic		
Manouvrier	Mesoskèles	Brachyskèles (mikroskèles)	Makroskèles		
		Brevilignes	Longilignes		
Viola	Normosplanchnic type	Megalosplanchnic type	Microsplanchnic type		

Pende		Hypervegetative biotype	Hypovegetative biotype		
Lombroso-Kurella					Primatoid anomalies (similarities to primates)
Sigaud	Muscular type	Digestive type	Respiratory type	Cerebral type	
Bryant	Herbivorous type		Carnivorous type		
Tandler	Hypertonic	Hypotonic			
Bauer		Arthritic habitus	Asthenic habitus		
Mills	Sthenic	Hypersthenic	Hyporsthenic, asthenic		
Brugsch	Normal chested	Wide chested	Narrow chested		
Kretschmer	Athletic type	Pyknic type	Asthenic type		Dysplastic types
Davenport	Medium	Fleshy	Slender biotype		
Bean		Mesontomorph	Hyperontomorph		
Stockard		Lateral type	Linear type		
Aschner	Normal	Broad	Slender		

CORRECTION

Medicine, Vol. V, No. 4, page 384, Table 1, add after Hippocrates:

	1	2	3	3a	4
Hallé-Husson (1821)	Tempéramens partiels:	Abdominale	Thoracique	Céphalique	
Rostan (1828)	Type musculaire	Digestif	Respiratoire	Cérébral	

(Hering (32)), is not practical and an unnecessary restriction. Without venturing to give a definition which would be theoretically binding, one may therefore, for the purpose of investigations like these, speak of the human constitution as the correlative unity of those morphological, physiological and psychobiological developments of the individual which are definitely more influenced by heredity than by environment.

COMPARISON OF CLASSIFICATIONS OF MORPHOLOGICAL TYPES

Attempts to describe morphological constitutional types have been made very frequently. None of these classifications, however, obtained final recognition (Wuth, l. c., p. 5). In many the very nomenclature shows the influence of physiognomical-symbolical ideas. Table 1 gives a cursory schematic survey of the various classifications of body types.[7]

Such a comparison of type constructions may give rise to the serious and not unjustified objection that these types originated at different ages and through totally heterogeneous methods, scientific and fanciful. Moreover it may appear inappropriate, or even unjust, to chart side by side the classical traditional types of Hippocrates, the romantic-symbolic system of Carus, the esthetic mythological analogies of the English anthropologist Walker, the dogmatic pathological "combinations" of de Giovanni, and the clinical anthropometric findings of Manouvrier, Viola, Brugsch, and Davenport, the brilliant intuitive divisions of Sigaud and his pupils which have been put to clinical use by Julius Bauer (5, 6), the pathological-anatomical observations of Rokitanski, Beneke and Kundrat, the anatomical types of Mills and Stockard, and the careful clinical descriptions of the psychiatrist Kretschmer. Nevertheless, it seemed worth while to collect and tabulate this not easily accessible material, because it seemed to illustrate several important considerations.

[7] Compiled from Aschner (2), Bauer (5), Bean (7), Brugsch (11), Bryant (12), Carus (13), Davenport (15), Dubreuil-Chambardel (16), de Giovanni (24), Hallé et Thillaye (29a), Kern (40), Kretschmer (44), Kundrat (48), Manouvrier (54), Mills (59), Pende (65), Rostan (73a), Stockard (85), Stratz (90). A number of these publications contain good illustrations. The illustrations show in many instances an unmistakable similarity between the types described by different authors. The photographs in the paper by Mills (1917) can be especially recommended. The photographs given by Bach (3) showing the comparison between the typical pyknic habitus and the physique of a professional wrestler are instructive.

The influence of physiognomical-symbolical notions and of clinical pathological observations, expressed in the nomenclature itself again and again, is striking, especially if it is taken into account that many of the authors did not know about one another. One of the fundamental mistakes of some of these classifications Kretschmer (44) has expressed very clearly, speaking of the types of Sigaud and his pupils: . . . . "their occasionally correct observations of detail are forced into a scheme which has been constructed purely speculatively, and at the basis of which—if we may express ourselves somewhat naïvely for the sake of clarity—lie the following ideas: there are (1) men of reason—these must have a big head; (2) eaters, these must have a fine belly; (3) acrobats, these must have splendid muscles; and (4) runners, these must have a fine pair of lungs" (p. 17).

The various types given in the groupings of different authors do not, of course, correspond with each other in the simple fashion schematically indicated on the chart. The question arises as to what extent they are comparable. There is no doubt that there is a thread of uniformity and that in a far-reaching way most of these classifications aim to express similar body forms. A few are almost identical. It is difficult to determine how close this correspondence is between the groupings of different authors, but the difficulty seems almost equally great when the attempt is made, with any amount of accuracy, to assign an individual to any one type of these morphological classifications.

OBSERVATIONAL AND ANTHROPOMETRIC METHODS IN GENERAL

With regard to methods the problem arises, whether in the systematic differentiation of recurrent forms of habitus of individuals one should rely on exact anthropometric measurements alone, on descriptions alone, or on a combination of both. This problem has a parallel in the history of racial anthropology. The extreme number of anthropometric measurements used by the Hungarian Török, who took five thousand measurements of the skull alone, was opposed by Sergi who proposed at one time to omit measurements altogether and rely on observations alone (Haddon (29), pp. 40–41). Experience with patients seems to indicate that the question is not such a simple one of either the one or the other or both. Even when a decision is made that inclusion of measurements in the working scheme is necessary

there still remains the greater problem of the choice of measurements to be used. This problem is not simplified by the fact that since investigations of this character are of a clinical rather than an anthropological nature, they must in all respects be adapted to the needs of the clinic.[8]

It is evidently possible to recognize recurring (i.e., typical) body forms from observations alone. The process of this recognition is a complicated one, an exact observation of certain details combined with the realization of the general impression of the individual in toto. In these observations the personal equation plays a not insignificant part. Undoubtedly this general unison impression is very useful and important, and requires training and technique.[9]

This procedure of judgment from observations has been very much criticized. Unfortunately the average medical clinician is not qualified to raise the objection that it is impossible to size up recurrent body types from general impression because as a rule he does not, during the examination of a patient, inspect the nude human body in an erect posture and at an appropriate distance. This is the only way to get a clear impression of the general body configuration type. The reason why the general practitioner as a rule does not do this is twofold. First, there are obvious practical obstacles with regard to the routine technique of medical examination. Secondly, clinicians are interested mostly in disturbances of body structure when there are more or less pronounced anomalies, as in pathological-endocrine types.

It is a different matter, however, to describe and recognize again more or less accurately certain body types and to prove their existence inductively, study their occurrence statistically and establish quantitative relations with other correlative signs such as the incidence of disease. Racial anthropology is confronted with similar but by no means identical problems. Races are distinguished by observational data, such as color of hair and skin, quantity of hair, shape of head and by anthropometric data relative to body proportions. Just those data

[8] We are greatly indebted to Dr. Adolph H. Schultz, Associate Professor of Physical Anthropology, Johns Hopkins University, for his never failing encouragement, advice and critique throughout the course of this study.

[9] We found it useful in the process of training ourselves to compare our observations reached independently and to discuss the discrepancies.

which are most useful in racial anthropology, however, namely complexion, form of hair, skull form, eye color and stature, are apparently of least importance in the differentiation of smaller non-racial groups of individuals such as groups afflicted with physical or mental disease.[10] For this purpose the study of the body proportions, and particularly the development of the body cavities, as de Giovanni has already recognized, is of first importance.

In psychiatry the morphology of the skull has often been regarded as of very particular significance. This primitive-symbolic belief that the morphology of one single part of the human body, quite apart from the rest of the body, must have an exceptional value as an indicator of mental faculties,[11] has probably also something to do with the age-old observation that microcephaly is not infrequently associated with idiocy. That the opposite is not true, namely that a large head does not denote a superior intelligence or yet a deficient one, was already known to Perdulcis (66), who states this explicitly.[12] There are only two points which give the morphology of the skull an exceptional position. One is the undoubted fact that there is a certain inter-relation between the development of the brain and the cranium. The scientifically well-established facts concerning this inter-relation are, however, very few. Reichardt's (73) finding of relatively small skull cavity and relatively high specific gravity of the cranium in schizophrenic, especially catatonic patients, is interesting and significant. The other point is that the formation of the skull may be,

[10] It is for considerations like these that the expression "disease races" used by Dr. G. W. Draper of New York seems unfortunate.

[11] Compare the head formation in statues of the Buddha. "It will also be noticed that the Buddha images have certain physical peculiarities, of which the most conspicuous is the ushnisha or protuberance on the top of the skull. Technically this appears to be derived from a western form of headdress, but in significance it is to be classed with the physical characters attributed by Indian physiognomists to the Superman, the Mahapurusha. This ushnisha serves to distinguish the Buddha figure from that of a mere Brother, for the head of the Bhikkhu is always shaved bare and without the Buddha's bump of wisdom" (Coomaraswamy (14), p. 331). The ushnisha does not grow only as an attribute of the Buddha with his growing wisdom and after his illumination, but is already present as a "constitutional sign" in all statues depicting Buddha as a child. See Adam (1), "Buddha as a child," plate 13.

[12] "Alioquin magnitudo capitis ut non bonam, sic non semper malam per se constitutionem indicat" (p. 62).

to some extent, an indicator of the formation of the rest of the body especially of the skeletal system, as was already known to Hippocrates. The teaching of Gall[13] has left behind it the belief that whatever correlation there may be between morphological and psychological traits must be expressed particularly clearly in the form of the skull. Peterson (67) regretted that we have no studies of the psychic histories of tribes who produced artificial malformation of their heads. It seems also significant in this connection that the mental reactions of otherwise crippled children and adults have received very little attention on the part of psychiatrists. De Giovanni and Viola were the first to prove that head and skull measurements can be neglected to some extent in constitutional researches. Borchardt (10) in a recent study agrees with this view.

Some authors, like Kretschmer (43), claim that description goes very much further than measurements, and that description alone is entirely sufficient to differentiate types. From anthropometric measurements he expects only a more exact formulation. He has supplemented his book with a comparison of the averages of absolute measurements,[14] a procedure anthropologically of little value. Mayer-Gross (57) has gone even further and believes that the study of morphological types in psychiatry should be independent of the exact methods of physical anthropology, and more a branch of psychology which can be compared with the study of other expressive phenomena like "graphology." This lack of distinction between physiognomic expression and mensurable morphology reminds one of the old temperament theories. Scheidt (76), on the other hand, emphasizes that these studies can have conclusive results only if they make use of the commonly accepted methods of physical anthropology, the uniformity

[13] It is not intended here to discuss the historical significance of Gall's teaching. That Gall's influence was not entirely a side-tracking one for neuropsychiatric research can be seen from the fact that one of the first cases of aphasia with an autopsy report of a lesion in the left frontal region of the brain was published by Thomas Hood in the "Phrenological Transactions," a journal devoted to Gall's teaching (Walter (91)).

[14] In physical anthropology absolute measurements such as head length or leg length are used as well as indices. An index expresses the percental relationship between two or more measurements. For example, the cephalic index $= \left(\frac{\text{breadth}}{\text{length}} \times 100\right)$. Index values are generally regarded as more significant than absolute values.

of which has been established more or less successfully at international congresses of physical anthropology. (Compare also Hrdlička: Anthropometry (34)). It is true that every new problem is apt to call for certain adaptations and alterations in technique, but the same principles should be followed.

The task of an investigation of morphological and psychopathological correlations[15] therefore resolves itself into the following questions: can recurrent typical body forms be determined by inductive anthropometric methods; can body types, differentiated by observation and description, be correlated with exact anthropometric data; are there any correlations between anthropometric and psychopathological findings?

THE MATERIAL OF THIS STUDY; PROCEDURE OF INVESTIGATION

The material of this study consists of sixty-five male patients chosen at random without any consideration of diagnosis or physical habitus. The patients were taken from the wards of the Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, and from the Spring Grove State Hospital, Catonsville, Maryland.[16] A series of thirty-one female patients who were observed and measured at the same time[17] is omitted from this study for the following reasons: anthropological data from female individuals are not strictly comparable with those of male individuals; dimensional changes of habitus at different ages are more marked in women than in men (Aschner (2), p. 125); the physical types described by Viola, Sigaud, and Kretschmer are more difficult to recognize in women (Bauer (6) mentions this for Sigaud's types, p. 1082); in very many anthropological studies women are omitted. It seemed that if there are significant differences in morphological habitus between individuals with different mental diseases, as Kretschmer has claimed, these differences should be at least indicated in an accurate study of a relatively small number. Since our material is diagnostically entirely unselected (except that the routine procedure of the Henry

[15] By "correlation" is understood "a more frequent concurrence of two phenomena than would be expected from a mere chance coördination" (Scheidt (75)).

[16] We acknowledge gratefully the coöperation of Dr. J. Percy Wade, Superintendent, Spring Grove State Hospital.

[17] A study of these female patients is in progress.

TABLE 2

NUMBER	NAME	DIAGNOSIS	PRE-PSYCHOTIC PERSONALITY	BODY TYPE	INDEX
		Predominantly affective reaction types			
1	E. L.	Hypochondriacal depression in intellectually debile person	Syntropic	Pyknoid	237
2	W. P.	Depression with unreality feelings following acute anxiety state		Pyknoid (athletic)	247
3	D. W.	Manic-depressive reaction type; depressive phase		Pyknoid (athletic)	302
4	M. C.	Depressive personality with circumscribed aggravations	Syntropic	Pyknic	212
5	R. R.	Agitated depression—third attack	Syntropic	Pyknoid	224
6	L. M.	Manic-depressive reaction type; depressive phase	Syntropic	Pyknic	182
7	G. N.	Mania; previous attack of chronic mania	Syntropic	Pyknic	206
8	J. H.	Manic-depressive reaction type; manic phase with schizophrenic elements (periods of inaccessibility, mutism, day dreaming and catalepsy); previous depressive attack		Asthenic (dysplastic features)	325
9	D. K.	Psychoneurotic depression-third attack		Pyknoid (athletic)	296
10	B. R.	Manic-depressive reaction type; fourth depressive attack	Syntropic	Pyknic	154
11	H. G.	Manic-depressive reaction type; hypomanic state. Previous manic-depressive cycles		Unclear	315
12	H. A.	Reactive depression with protracted fatigue symptoms		Pyknoid (athletic)	255
13	R. M.	Manic-depressive reaction type; depressive phase	Syntropic	Athletic	266
14	H. D.	Depression with somatic delusions; previous psychotic attack at fourteen		Unclear	257
15	T. D.	Agitated depression, second attack; cerebral arteriosclerosis	Syntropic	Pyknoid	262
16	W. F.	Manic-depressive reaction type; second depressive attack	Syntropic	Pyknic	167
17	T. B.	Manic-depressive reaction type; depressive phase. Previous manic-depressive cycles	Syntropic	Pyknoid (athletic)	276
18	E. F.	Depression with schizophrenic elements (auditory and visual hallucinatory experiences and ideas of reference)	Syntropic	Asthenic	291

19	M. J.	Manic excitement with schizophrenic elements (feelings of influence, revelation experiences, hallucinations of God's voice through canary bird)		Asthenic-athletic M-F	326
		Predominantly schizophrenic reaction types			
20	N. S.	Schizophrenic reaction type	Idiotropic	Asthenic (dysplastic features)	313
21	H. T.	Schizophrenic paranoid reaction type	Idiotropic	Asthenic-athletic M–F	299
22	W. S.	Schizophrenic paranoid reaction type. Catatonic stupor		Athletic	306
23	B. S.	Catatonic stupor	Idiotropic	Asthenic-athletic M-F	314
24	C. K.	Anxiety state with schizophrenic paranoid development		Pyknoid (athletic)	199
25	J. H.	Schizophrenic paranoid reaction type	Idiotropic	Asthenic-athletic M-F	292
26	R. C.	Schizophrenic reaction type	Idiotropic	Unclear	267
27	J. C.	Schizophrenic paranoid reaction type with early catatonic phase		Athletic (dysplastic features)	298
28	H. B.	Schizophrenia—simple type	Idiotropic	Asthenic (dysplastic features)	337
29	M. B.	Schizophrenic paranoid reaction type	Idiotropic	Athletic	248
30	E. W.	Schizophrenic reaction type	Idiotropic	Asthenic-athletic M-F (dysplastic features)	270
31	G. G.	Schizophrenic paranoid reaction type		Asthenic (dysplastic features)	315
32	J. H.	Schizophrenic reaction type		Asthenic-athletic M-F	252
33	G. W.	Schizophrenic paranoid reaction type		Athletic	268
34	H. O.	Schizophrenic reaction type		Asthenic-athletic M-F	260
35	A. B.	Schizophrenic reaction type	Idiotropic	Athletic	304
36	E. S.	Schizophrenic paranoid reaction type	Idiotropic	Asthenic-athletic M-F (dysplastic features)	333
37	C. F.	Schizophrenic reaction type; scattered deterioration	Idiotropic	Pyknoid (asthenic)	242
38	G. N.	Schizophrenic reaction type; scattered deterioration	Idiotropic	Asthenic (dysplastic features)	282
39	A. G.	Schizophrenic reaction type	Idiotropic	Pyknoid (athletic)	294
40	W. B.	Schizophrenic reaction type		Pyknoid (athletic)	229
41	J. H.	Schizophrenic reaction type		Pyknic (dysplastic features)	178

TABLE 2—*Continued*

NUMBER	NAME	DIAGNOSIS	PRE-PSYCHOTIC PERSONALITY	BODY TYPE	INDEX
		Predominantly schizophrenic reaction types—Continued			
42	A. B.	Schizophrenic reaction type; social recovery	Idiotropic	Athletic	263
43	R. D.	Schizophrenia—simple type	Idiotropic	Asthenic-athletic M-F	302
44	M. S.	Schizophrenic reaction type		Athletic (dysplastic features)	254
		Psychopathic personalities + psychoneuroses			
45	R. O.	Psychopathic personality; alcoholic hallucinosis		Asthenic-athletic M-F	284
46	B. R.	Chronic alcoholism		Athletic	299
47	J. G.	Intellectual debility; hysteria	Idiotropic	Pyknoid (asthenic)	271
48	W. B.	Constitutional psychopathic inferiority; emotional instability		Asthenic-athletic M-F	311
49	J. H.	Post-traumatic hypochondriasis		Pyknic	224
50	F. W.	Supra-orbital pain on psychogenic basis	Syntropic	Pyknoid	243
51	J. M.	Feeblemindedness		Asthenic-athletic M-F	296
52	W. N.	Constitutional psychopathic inferiority; emotional instability; alcoholism		Asthenic-athletic M-F	282
53	L. S.	Atypical tabes (?); hysterical reaction	Syntropic	Pyknic	153
54	J. A.	Feeblemindedness with schizophrenic-like psychotic episodes		Pyknic	197
55	R. W.	Constitutional psychopathic inferiority; antisocial tendencies		Pyknoid (athletic)	242
		Organic reaction types			
56	C. P.	Epilepsy		Asthenic	298
57	C. D.	Dementia Paralytica		Pyknoid (athletic)	227
58	F. S.	Dementia Paralytica with previous depressive attack		Pyknoid (dysplastic features)	234
59	C. A.	Cerebral lues		Pyknic	244
60	C. C.	Epilepsy	Syntropic	Pyknic	186
61	J. R.	Epilepsy		Pyknoid (athletic)	243
62	G. I.	Metencephalitic Parkinson syndrome	Syntropic	Pyknic	199
63	A. S.	Dementia paralytica		Pyknic	194
64	L. B.	Depressive psychosis following encephalitis lethargica		Asthenic-athletic M-F	276
65	G. A.	Left hemiplegia		Asthenic-athletic M-F	304

Phipps Psychiatric Clinic prefers early and diagnostically complicated or therapeutically promising cases, and the material from the State Hospital naturally tends to a predominance of chronic cases), a variety of diagnostic groups is represented. Kretschmer's book and the studies of most investigators based on his book deal only with manic-depressive and schizophrenic cases.

The patients are classified in four diagnostic groups:

1. Predominantly affective (manic-depressive) reaction types
2. Predominantly schizophrenic reaction types
3. Organic reaction types
4. Psychopathic personalities and psychoneuroses

This division was made purely for practical purposes in order to avoid a larger number of diagnostic groups with only a few individuals in each group.

In the first group are placed nineteen cases of affective psychoses,[18] both cases with more or less typical course of manic-depressive psychosis and cases which, though predominantly affective, present certain other features such as schizophrenic-like reactions. The special diagnosis of each case is given in table 2. In the same way there are in the second group twenty-five predominantly schizophrenic patients with the special diagnosis indicated. In the third group are eleven cases of psychopathic personalities and psychoneuroses, including also cases of chronic alcoholism, a diagnostically not quite clear case of post-traumatic hypochondriasis, and, merely for the practical considerations mentioned above, cases of feeblemindedness. In the fourth group are ten cases of organic reaction type including epilepsy.

The nosologically indefinite arrangement of cases in the last two groups is of little significance inasmuch as the emphasis of the study is naturally on the two more constitutional reaction types of the first two groups. In these first two groups it seemed of special interest to see whether those cases for the sake of which the qualification "predominantly" was introduced showed any special morphological features. The designation of manic-depressive psychosis with schizo-

[18] This term is used in a similar meaning by Kahn in a recent genealogical-clinical study (37).

phrenic features is not intended to indicate a co-existence or blending of different "hereditary units," but is employed merely in order to do justice to the clinical facts of the individual case. It may be added that more or less typical manic-depressive reaction types according to any strict terminology are relatively rare in hospitals, as compared with schizophrenic psychoses, so that serious doubt has been cast on the diagnostic conscientiousness of those investigators who have studied in a relatively short time the same large number of manic-depressive and schizophrenic psychoses (Kolle (42), p. 605).

In the course of the investigation three kinds of data were kept entirely separate, namely,

1. Psychiatric data
2. Data obtained in form of observations
3. Anthropometric data

Each set of data for each patient was separately numbered, and the psychiatric, observational, and anthropometric data not compared until the final correlation. The formulation of the psychiatric diagnosis was written down in the present form (see table 2) before the anthropometric valuations were completed.

DESCRIPTION OF MORPHOLOGICAL TYPES USED

In the classification of morphological types we followed mainly the descriptions given by Kretschmer (44). From the observation of our patients and from exact anthropometric data (to be discussed later) a group of patients seemed to be distinct enough to be classed together as a sub-type or transition type which we shall refer to as pyknoid. The chief characteristics of these types are as follows:

1. The pyknic individual (plate 1) has in profile a round head, the contour of the occiput, top of the skull and profile line impressing one often as a more or less clear circle. The outline of the head is soft without any sharply prominent parts (like nose or chin). The face has the form of a pentagon or a shield (see fig. 3, I and II) or in many cases a form between the two. It is always definitely broad and soft, quite apart from the fact that it is usually fleshy, especially as regards the nose. There can be no doubt that in the general aspect of the

face the impression of a certain harmoniousness and balance is also conveyed by certain physiognomical-psychological characteristics which evidently may be a source of error. The neck is short and full, with the head set a little forward on smoothly rounded shoulders. The line from the tip of the chin to the suprasternal notch is characteristic, inasmuch as it tends to be not an angle but a smoothly sloping or even a straight line.[19] The hair line usually borders the forehead in a regular unindented curve. Apparently the pyknic type has a tendency to baldness, and when this occurs it seems to be electively regular in outline and with a smooth shiny surface. The trunk is thick set and appears barrel-shaped, with the chest broadening

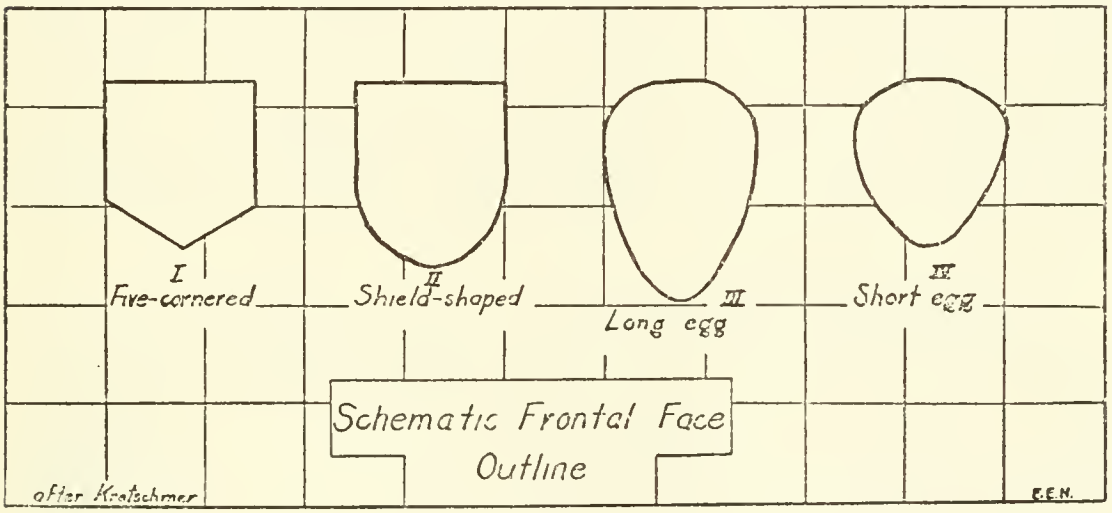

FIG. 3. SCHEMATIC REPRESENTATION OF THE FACIAL OUTLINE

toward the lower part of the body. One might say that the body cavities (head, chest, abdomen) tend to a voluminous development. The limbs are relatively short, as are also the hands. The panniculus adiposus is well developed and the color of the skin is good. The pyknic habitus is most definitely pronounced in early middle age.

1a. There are undoubtedly pyknic admixtures in other cases and in order to do justice to these frequent not so distinct types which

[19] The passage in Kretschmer's book (English translation), p. 29 (44), where this trait is described has been wrongly translated so that the meaning has been destroyed. The translator evidently confounded the German word "Sternalende" which means end of the sternum, with the word "Stirne," meaning forehead. This passage has been quoted uncorrected in a number of reviews of the subject.

impress one as having definitely pyknic components we propose to speak of a pyknoid habitus.

2. The athletic type is characterized by a strong development of the locomotor apparatus so that the bones and muscles stand out in plastic relief. The heavy development of the skeleton is most easily recognized in the clavicles. The neck is strong and the trapezius muscles typically very well developed. The shoulders are large and the trunk tapers down from the broad shoulder girdle to the relatively narrow pelvis, so that the trunk outline from the front can be schematically designated as a trapezoid.[20] The limbs are relatively long, the hands and feet are large and the fingers often of a blunt, thick and "acromegaloid" character. The hair is abundant and the hair line tends to grow in a peak in the center of the forehead, receding in bay-like manner on either side toward the temples. The face has a long egg[21] form (see fig. 3, III). A line which would connect the upper forehead, tip of nose, and chin would be a gentle curve. There is a variant, if one may speak of such, which is on the whole more plump and massive.

3. The asthenic habitus[22] (plate 2) is characterized by the general impression of a deficiency in volume combined with an average unlessened length, so that these individuals impress one as being taller than they really are. The thorax is long and flat and the shoulders narrow, and the trunk can be schematically described as cylindrical. The legs and arms are long and thin with lean muscles. The face is of short egg form (see fig. 3, IV) and may show hypoplasia in varying degree. The skin tends to be pale, thin, and with but scant fat. The profile is usually markedly irregular. The line which would connect upper forehead, tip of nose and chin tends to be angular on account of the disproportion between the long nose (which seems sharp on account of lack of fatty upholstering) and the small mandible. When the hypoplasia of the jaw is not so marked there may be noted simply a long nosed profile. The neck is thin and seems elongated. The

[20] The Hindus compared this type of trunk with the head of a cow (Tagore (87)).

[21] The Hindus compared a type of face with the egg of a chicken (see Tagore (87), p. 24).

[22] This term is used here not in a clinical but in a purely morphological sense. Stiller (84) described a "morbus asthenicus" in which he included almost all deviations from the normal constitution. Compare also Zerner (94).

body hair is scanty and these individuals may also tend to baldness, but with an irregular outline and a more or less wrinkled surface. Other individuals who on account of some signs might be subsumed under this type have wider shoulders accompanying the usual flat chest.

4. Dysplastic body forms show stunted growth either of the whole body or parts, i.e., very small nose or minute hands and feet or dysgenital (eunuchoid) features (long extremities, broad pelvis, scanty hair, unusual distribution of fat). The undergrowth of the nose, together with a certain softness of the facial outline, may give the face a pseudo-pyknic appearance, as can also eunuchoid obesity of the trunk. However, this habitus can be easily distinguished from the pyknic type by attention to details. On account of the underdevelopment of the middle part of the face, the line connecting upper forehead, tip of nose and chin may approach a straight line. On the whole this group of individuals with dysplastic features contains all the very marked variations from the most common types.

METHODS OF OBSERVATION AND DESCRIPTION

The following observations were recorded for each patient.[23]

1. Fractures
2. Right-handed
3. Head form
4. Face shape
5. Profile: nose
6. Eyes: color, set
7. Ear
8. Hair form
9. Hair color
10. Baldness
11. Neck
12. Larynx
13. Thyroid
14. Trunk outline
15. Fat: deposits: masculine, feminine, infantile
16. Musculature
17. Scapulae
18. Hair amount: scalp, eye-brows, chin, cheeks, trunk, arms, legs, pubes, axilla
19. Hair texture
20. Skin: color, texture, moisture
21. Moles
22. Freckles
23. Wrists and ankles
24. Foot arch
25. Fingers: pointed or blunt
26. Nails: lunuli, curvature
27. Testes: sexual anomalies

In order to avoid the usual descriptive terminology which does not lend itself readily to later analysis, comparison and classification, an

[23] A complete medical physical examination was done on each patient.

attempt was made to make it more simple and accurate. First a system of + and − signs was used to indicate average (+), more than average (++), and excessive (+++), with (+−) less than average and (−) absent. This was more concise than describing the amount of hair and fat in adjectives and was more convenient in the final analysis. Secondly, diagrams were used to obtain a uniformity of detailed observations, with the personal element of the observer reduced as much as possible, and therefore with a greater possibility for the checking up of results by other observers.

The following diagrams were used. The schematic facial outline types are those of Kretschmer (fig. 3). The trunk outlines are adapted from photographs of patients, illustrating recurring conditions (fig. 4). The outlines of face and head are recorded similarly after observation of a number of individuals with a selection of only the most elementary differences in alignment of features (fig. 5, rear profile and top of head from above; fig. 6, forehead, lips and chin). Figure 7 shows diagrams of hands. Diagrams were also used to aid in recording observations regarding hair form, nose, shoulder blades and ear shape. The diagram for hair form was taken from Sullivan (86) after Martin. The diagrams for nose and ear shape were adapted directly from Martin (56).

In about one-third of the cases photographs were taken showing front view of head and chest, profile of head and chest, and trunk in one or two views. These were numbered and the morphological types recorded independently from the other observations.[24] These diagnoses agreed with the classification made from observation of the patients.

BODY TYPES FROM OBSERVATION

Our material is too small to yield any conclusive results as to the frequency of occurrence of physical types. Moreover, the percentages of these types in the general population are not sufficiently known. However, since it is a true random sample which contains representatives of the different type groups, a survey of the percentages is not without interest.

[24] We are indebted to Dr. O. Wuth, Associate in Psychiatry, Johns Hopkins University, for diagnosing these photographs for us according to morphological types independently and without any other knowledge of the material.

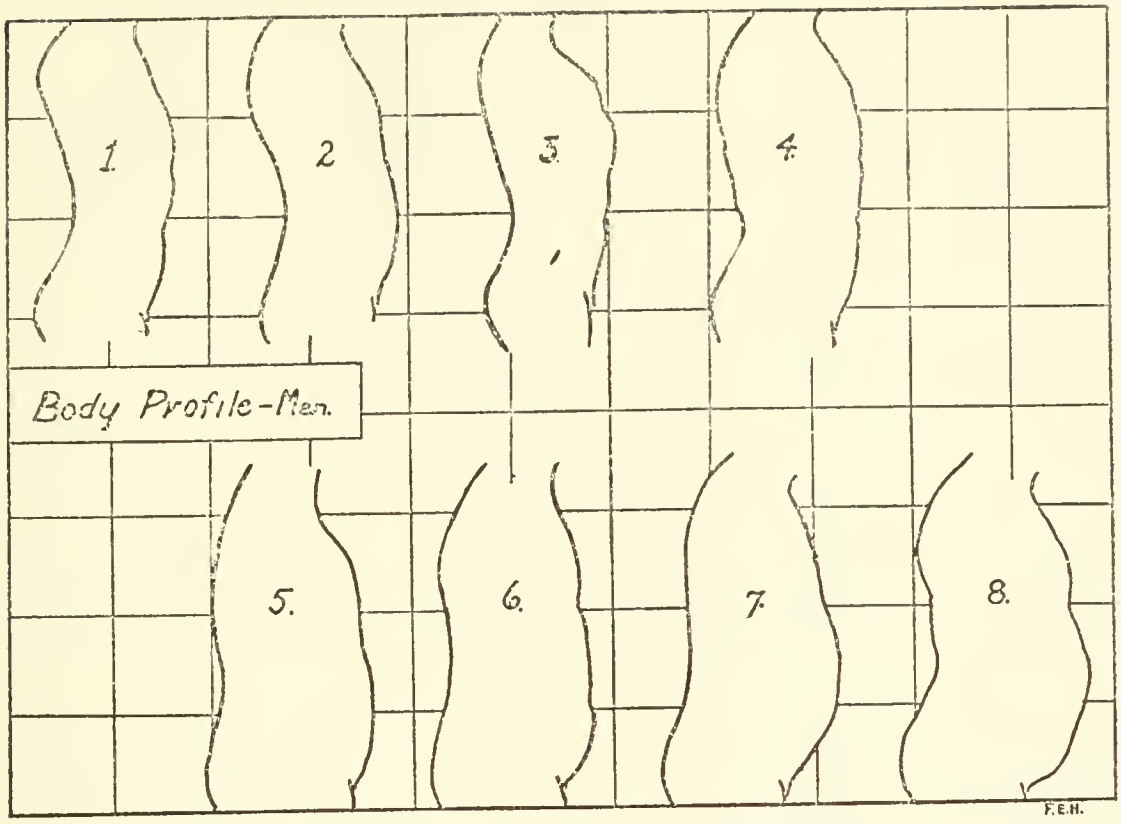

FIG. 4. SCHEMATIC REPRESENTATION OF TRUNK PROFILE

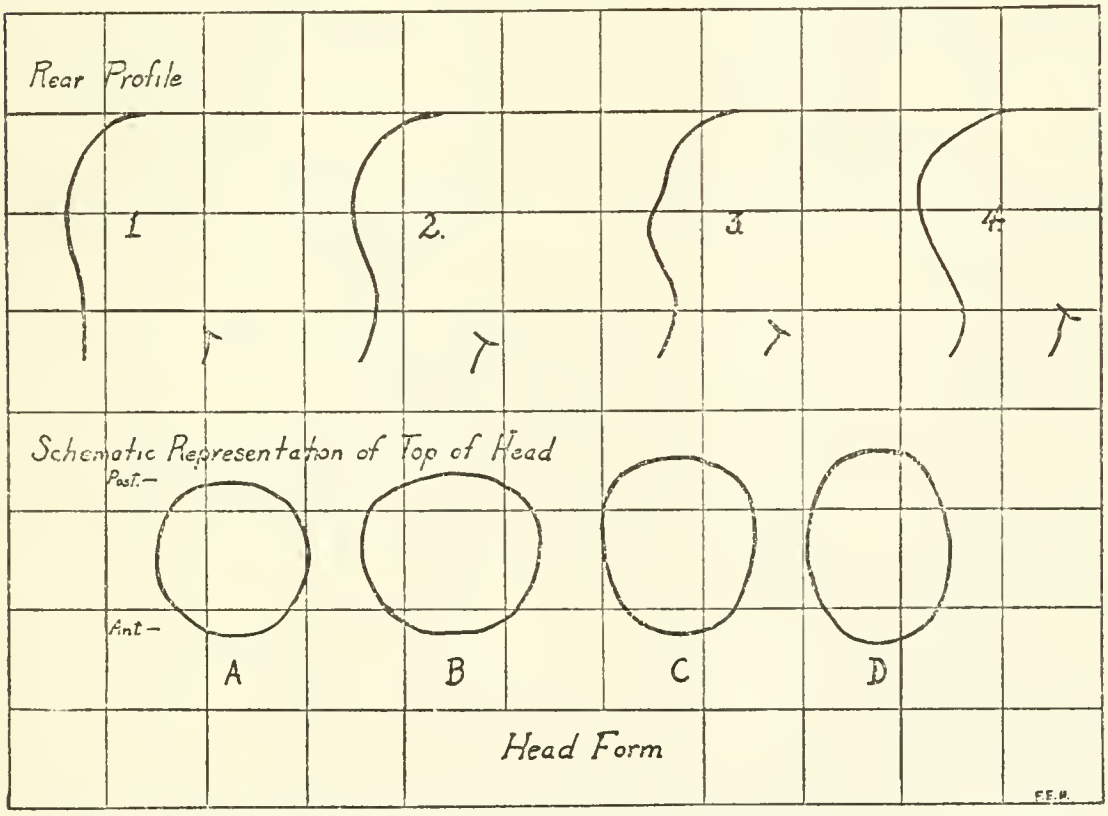

FIG. 5. SCHEMATIC REPRESENTATION OF HEAD FORM (REAR PROFILE AND TOP OF HEAD)

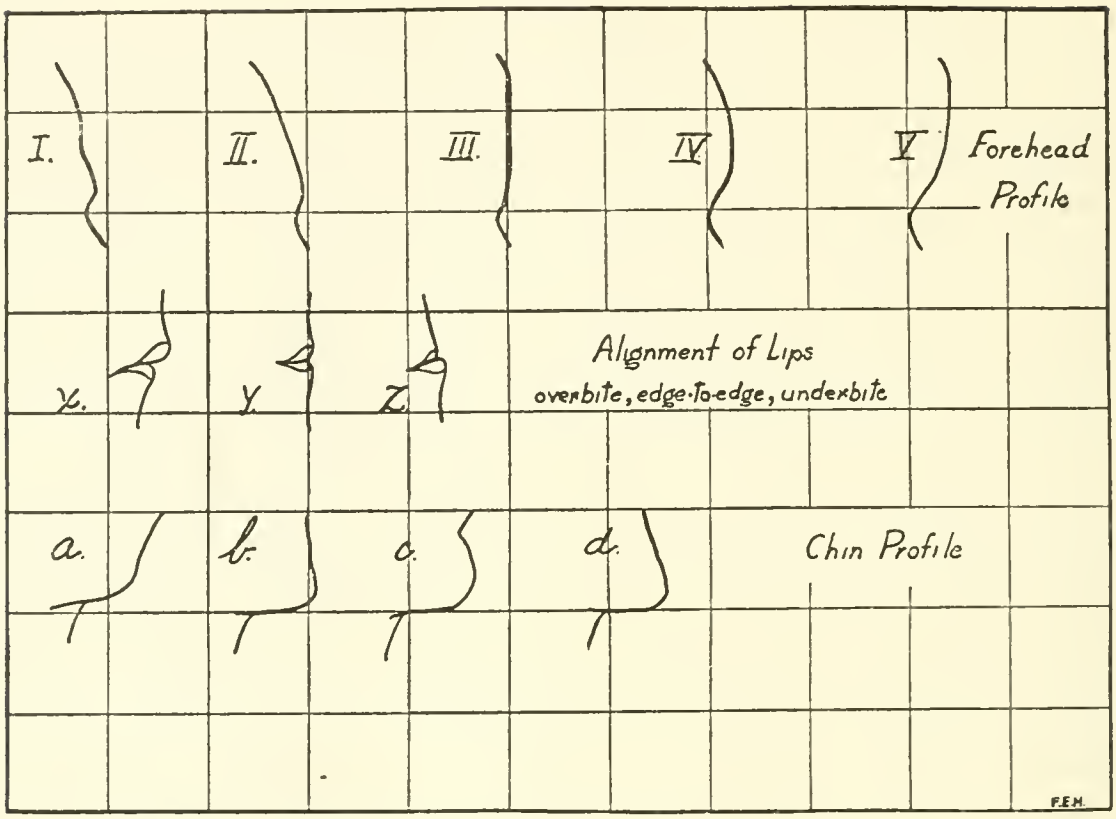

FIG. 6. SCHEMATIC REPRESENTATION OF FOREHEAD, ALIGNMENT OF LIPS AND CHIN PROFILE

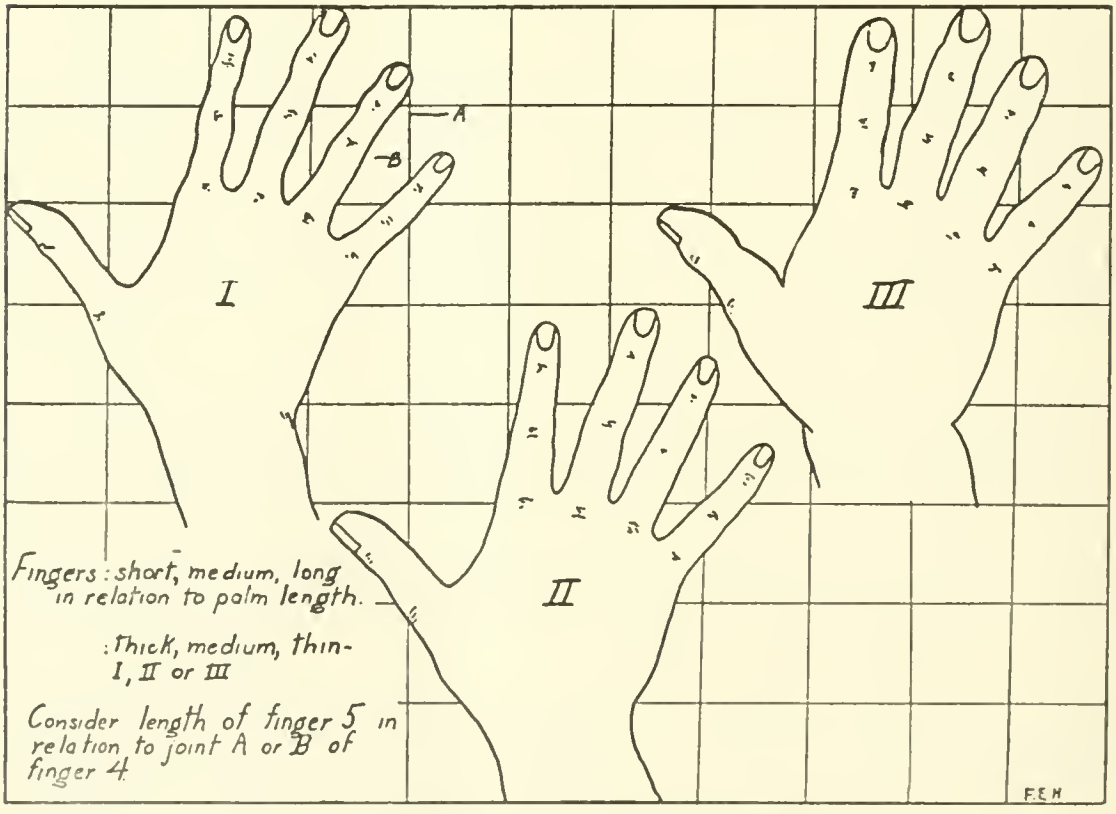
A
B
I
II
III
Fingers: short, medium, long in relation to palm length.
: thick, medium, thin- I, II or III
Consider length of finger 5 in relation to joint A or B of finger 4
F.E.H.

FIG. 7. SCHEMATIC CLASSIFICATION OF HANDS

Percentages of types in 65 cases

	per cent
13 pyknics	20.0
7 asthenics	10.8
9 athletics	13.8
18 pyknoid	27.7
15 asthenic-athletic mixed forms	23.0
3 unclear	4.6

or

Pyknic forms		20.0
Pyknoid	27.7	69.1
Athletic	13.8	
Asthenic-Athletic	23.0	
Unclear	4.6	
Asthenic		10.8

These percentages cannot claim any special significance. However, it is interesting to know that they may suggest a normal distribution curve of symmetrical character (see later).

These percentages include among the others those cases with dysplastic features. Cases with dysplastic features can be subsumed under some of the other types too. They were therefore included in the above tables. Twelve of our cases show dysplastic characteristics, in facial outline, distribution of fat and hair or in general proportions. Of these twelve cases, eleven show marked or suggested feminine distribution of pubic hair among other dysplastic features. Only one has normal distribution of pubic hair (no. 20). His dysplastic features consist mainly of marked disproportion of limb length and stature. The main other findings in his case were: mydriasis; eyebrows a little scanty lateralward; small mouth; palate narrow; large hands and long fingers; cyanotic hands and sweaty palms; narrow costal angle; liver dullness not quite two finger breadths below costal margin in right mammary line; gonads normal in size; hypotrichosis of arms and legs, especially thighs; eye grounds: very large physiological cupping; discs of good color; lymphocytosis 43 per cent (Dr. Thomas P. Sprunt).

With the exception of one case in the pyknic group (no. 41) and one case in the pyknoid group (no. 58) all of these dysplastic features occur in cases of asthenic, athletic, or asthenic-athletic mixed forms. None of them occur in the three unclear cases. Dividing cases into pyknic, athletic, asthenic, and dysplastic groups, as Kretschmer does, involves the use of two different principles of classification. This

is of importance because a separate correlation of the dysplastic group with psychiatric diagnoses is carried out. The percentage of dysplastic cases was 18%.

ANTHROPOMETRIC METHODS

The third series of independent data consists of anthropometric measurements. Fifty-three of these measurements were taken on each patient according to the technique described by Martin (55) (56) and Hrdlička (34).[25]

The following table gives the list of absolute measurements taken:[26]

1. Weight (kilograms)
2. Span
3. Stature
4. Xiphoid height
5. Symphysis height
6. Anterior iliac spine height
7. Tibiale height
8. Femur height
9. Malleolus height
10. Sitting height
11. Trunk height
12. Horizontal head circumference
13. Horizontal chest circumference: maximum inspiration, rest, maximum expiration
14. Greatest arm circumference
15. Smallest arm circumference
16. Greatest thigh circumference
17. Greatest calf circumference
18. Smallest leg circumference
19. Chest length
20. Subcostal angle
21. Bi-acromial diameter
22. Bi-iliac diameter
23. Greatest hip diameter
24. Transverse chest diameter
25. Sagittal chest diameter
26. Arm length
27. Humerus length
28. Radius length
29. Hand length
30. Hand breadth
31. Palm length
32. Finger length
33. Nail length
34. Nail breadth
35. Maximum head length
36. Maximum head breadth
37. Bizygomatic diameter
38. Bigonial diameter
39. Head height
40. Total face height
41. Upper face height
42. Nasal height
43. Nasal breadth
44. Ear length
45. Ear breadth
46. Ascending ramus length
47. Horizontal ramus length
48. Mandible length
49. Gonial angle
50. Interpupillary space
51. Interocular breadth
52. Palpebral length
53. Palpebral breadth

[25] The absolute measurements were taken in the clinical laboratory of the Henry Phipps Psychiatric Clinic with a set of instruments devised by the late Professor Martin of Munich. In connection with these measurements a series of physiological tests was done by Dr. R. S. Lyman which is being worked out independently.

[26] The anatomical terminology is given in preference to the anthropological one, which uses terms such as symphysion, nasion, etc., which are not so familiar to the medical reader.

Thirty-seven different indices were calculated and analyzed in order to find out whether inductively, from a statistical evaluation of anthropometric measurements alone, a grouping of outstanding body types would emerge. These indices are given by Martin (55). The procedure in using absolute measurements and indices was made in two ways. First, frequency distribution curves[27] were plotted for both absolute and proportional measurements. Although the number of cases was too small to expect conclusive results from a frequency distribution, at least an indication of a trimodal frequency distribution might have been expected if the pyknic, athletic and asthenic types were sufficiently distinct. Or further there existed the possibility that if the pyknic and non-pyknic body forms were antagonistic types,

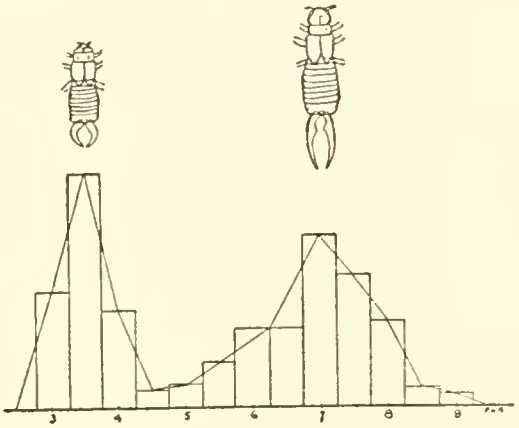

FIG. 8. BIMODAL POLYGON ILLUSTRATING DIFFERENTIATION OF TWO SPECIES PLOTTED FROM DATA ON EARWIG
(From Bateson and Johannsen)

a bimodal frequency distribution curve (cf. fig. 8) might reveal a suggestion as to the distribution of these two types. No indication of bimodal or trimodal frequency distribution was obtained.

Secondly, the measurements obtained were compared with the averages and ranges of variation for the pyknic, asthenic and athletic types as given by Henckel (30) (31). An analysis was made of the measurements and indices of each patient of the group of this study

[27] A normal frequency distribution curve, that is to say a curve of chance, is a symmetrical one in which the mode, i.e., the highest point in the curve, and the mean, i.e., the arithmetic average, occur on the same ordinate. A socalled biomodal curve is one which has two peaks and, when representing a sufficiently large sample of data, may represent the occurrence of two separate elements in the data considered (see fig. 8). Similarly the trimodal curve has three distinct peaks and may indicate three types or elements.

in comparison with the figures given by Henckel as the average, highest and lowest values for each type, as found in his group of cases. This study yielded two negative results. First, on the basis of the measurements used by Henckel, it was not possible to assign the individual cases definitely to one or another of the type groups; secondly, no absolute measurements or indices were indicated as selectively significant in assigning cases in respect to body type, with the exception of the Pignet Index (to be discussed later).

The following is the list of indices which were calculated:

1. Weight—stature
2. Stature—span
3. Sitting height—stature
4. Trunk height—stature
5. Trunk height—span
6. Anterior-posterior diameter—lateral diameter chest
7. Anterior-posterior diameter—chest length
8. Lateral diameter—chest length
9. Chest circumference—chest length
10. Chest circumference—stature
11. Bi-acromial diameter—trunk height
12. Bi-iliac diameter—biacromial diameter
13. Bi-iliac diameter—trunk height
14. Arm length—leg length
15. Arm length—trunk height
16. Humerus length—femur length
17. Tibial length—femur length
18. Trunk height—leg length
19. Radius length—humerus length
20. Finger length—palm length
21. Hand length—radius length
22. Hand breadth—hand length
23. Nail breadth—nail length
24. Leg length—thigh length
25. Cephalic breadth—cephalic length
26. Cephalic height—cephalic length
27. Total face height—bizygomatic diameter
28. Upper face height—bizygomatic diameter
29. Interpupillary space—bizygomatic diameter
30. Nasal breadth—nasal length
31. Ear breadth—ear length
32. Cephalic height—cephalic breadth
33. Arm length in per cent stature
34. Leg length in per cent stature
35. Korperfülle Index (Rohrer)
36. Pignet Index
37. Morphologic Index

METHOD OF CORRELATING OBSERVATION TYPES WITH ONE INDEX; "CONSTITUTIONAL INDICES;" WEIGHT

The conclusion was therefore reached that these body types could probably not yet be found inductively by anthropometric methods or expressed accurately in anthropometric terms of body proportions. This does not mean that anthropometric methods themselves are not applicable for the distinction of these types, for as in racial anthropology the observation types can be used as a starting point and then correlated with anthropometric measurements. Absolute measurements, such as Kretschmer used in his book, are the least conclusive for such a procedure, for slight differences in absolute measurements may be found in any two groups of individuals, whether they be admissions to a hospital or admissions to a club. In considering the possibility of an index, therefore, which would define the physical habitus of an individual, it is necessary to keep in mind the fact that such a so-called constitutional index should represent the characteristic morphology of the individual as a whole, as contrasted with such indices as the cephalic index, which states the proportion between two measurements of only one part of the body.

As de Giovanni has already pointed out, the impression of the existence of morphological types is dependent on the different size of the body cavities and their relation to the limb length. Naccarati's morphological index is evidently aimed at just this proportional relationship (62). This index, however, is not suitable for psychiatric patients and its accuracy in other constitutional studies may be questioned since in the one index is involved the use of ten different measurements. The fact that two of these measurements may be influenced by subcutaneous fat, variable in most mental patients, increases the opportunity for error. Slight errors may be much magnified in the final result, since nine measurements must be multiplied before being subsequently added. A theoretical criticism of this index might also be raised on account of its form, the mathematical propriety of dividing the product of three measurements by the sum of two others being open to question. More explicit information as to the technique of taking the measurements for this index would be necessary. Measuring a diameter "at the level of the fourth rib"

without further instructions becomes difficult when it is considered that the fourth rib curves in three dimensions, and varies in position during breathing.[28]

The variations in weight during psychoses rule out for exact evaluation of the physical constitution of psychiatric patients three other indices which have been used as an index of the physical constitution, namely:

1. The Becher-Lenhoff Index: (Martin (55), p. 27 $\frac{\text{Length of anterior trunk wall}}{\text{Waist circumference}} \times 100$

2. The height-weight index of build (Buffon, Rohrer, Bardeen (4): $\frac{\text{Weight}}{\text{Stature}^3}$

3. The Pignet Index (68): Stature (cm.) − [(chest circumference (cm.) + weight (kgm.)]

The Becher-Lenhoff Index contains as one of its components the waist circumference which is dependent on the state of nutrition of the individual. The Rohrer Index and the Pignet Index contain weight itself. Of these two the Pignet Index requires particular attention since it has been quoted by Kretschmer as an anthropometric confirmation of his findings. Kretschmer also reproduces the table obtained by Henckel (Kehrer and Kretschmer (39), p. 179). Three objections can be raised against the Pignet Index as an accurate anthropometric method for the study of the morphological constitution. First it compares a linear with a non-linear measurement (weight); secondly, its accuracy is not beyond doubt since one centimeter is regarded as equivalent to 1 kgm. of body weight. A variation of 1 cm. in chest circumference can easily occur in two successive examinations. More important than these two is the weight factor. Weight, which is a component of the index, changes considerably in the course of many psychoses so that evidently the index gives different values at different stages of the disease. The weight of both manic-depressive and schizophrenic patients tends to be influenced very much dur-

[28] This index has been used by Dr. F. Shaw (80) in a recent study. His data are not significant since no figures regarding the range of variations are given. Aside from this fact, the significance he attaches to the biomodal curve obtained from a frequency distribution of his index values may be questioned. The difference between the two peaks of this curve (which he interprets as definite evidence of the existence of two body types) is numerically one hundred points. Naccarati (62) states that "an error of one-half centimeter plus or minus would cause a change of fifty counts in the index" (p. 21).

ing the course of the psychosis. In Henckel's study (30) (31) this error is considerably increased by the fact that while his schizophrenic patients were examined during their stay in the hospital (i.e., during the psychosis) a large number of his manic-depressive patients were examined after recovery when the patients were asked to return to the dispensary. Thus the evaluation of the Pignet Index in manic-depressive cases was influenced by the fact that the majority of the patients with manic-depressive psychoses as a rule gain weight in the course of recovery, and some in fact to a very considerable degree and with great regularity. Kolle has clearly recognized this error without, however, finding a means of evading it (Kolle (41)). He substituted the weight on admission for the weight taken at the time of examination. This method neglects the fact that the period of illness before the patient seeks admission to the hospital varies of course greatly in different patients and they may, and often do, lose a considerable amount of weight at that time. Figure 11 illustrates the possible variations of weight during the course of recovery. The patient (no. 10) gained 74 pounds (or 33.8 kgm.) from the date of his admission on June 30 to March 15. His best weight was slightly more than 250 pounds about two years before admission. This patient was from observation a clear pyknic type, not very conspicuously fat; he was a well-built actor.

Henckel assumed that the following averages and ranges of variation of Pignet Index values correspond with the morphological types in mental patients.

	AVERAGE	RANGE OF VARIATION
Asthenic	+34.3	(23.0– 54.2)
Athletic	+11.9	(10.6– 21.8)
Pyknic	−5.5	(−34.8–+12.2)

Three examples may be sufficient to show the inaccuracy of this method.

1. Patient no. 15. Weight on December 22, 45.9 kgm. His Pignet Index was 33.7. According to Henckel's figures this should signify an asthenic constitution. During the course of recovery from his depression he gained weight and reached by May 12 the weight of 60.0 kgm. This influences

the Pignet Index to the value of 19.6, which according to Henckel would signify an athletic constitution (see fig. 9).

2. Patient no. 6. Weight on March 4, 59.0 kgm. His Pignet Index was

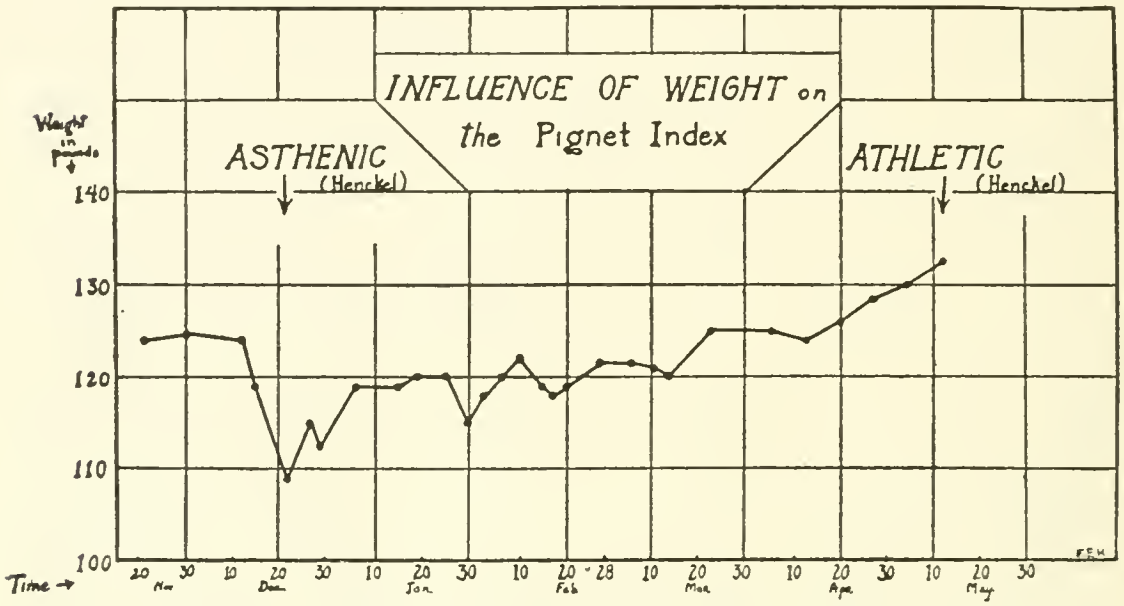

FIG. 9. INFLUENCE OF WEIGHT ON THE PIGNET INDEX I

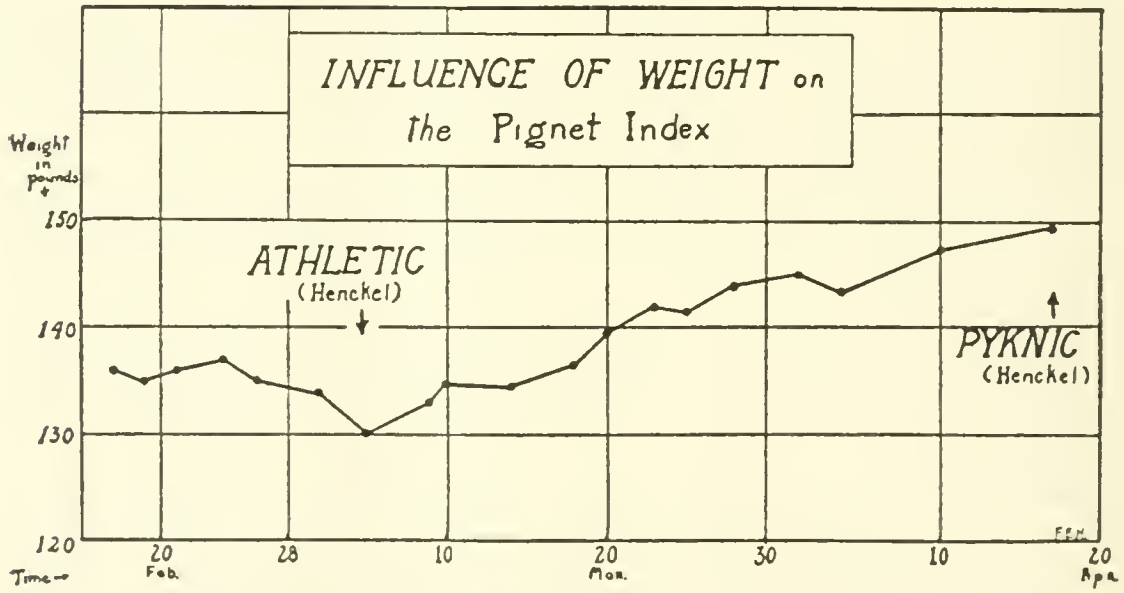

FIG. 10. INFLUENCE OF WEIGHT ON THE PIGNET INDEX II

+18.7. This figure would place him, according to Henckel's figures, in the athletic type group. By April 17 he had gained during the course of recovery from his depression to the extent of weighing 68.0 kgm. His Pignet Index calculated for April 17 was therefore +9.7. In other words,

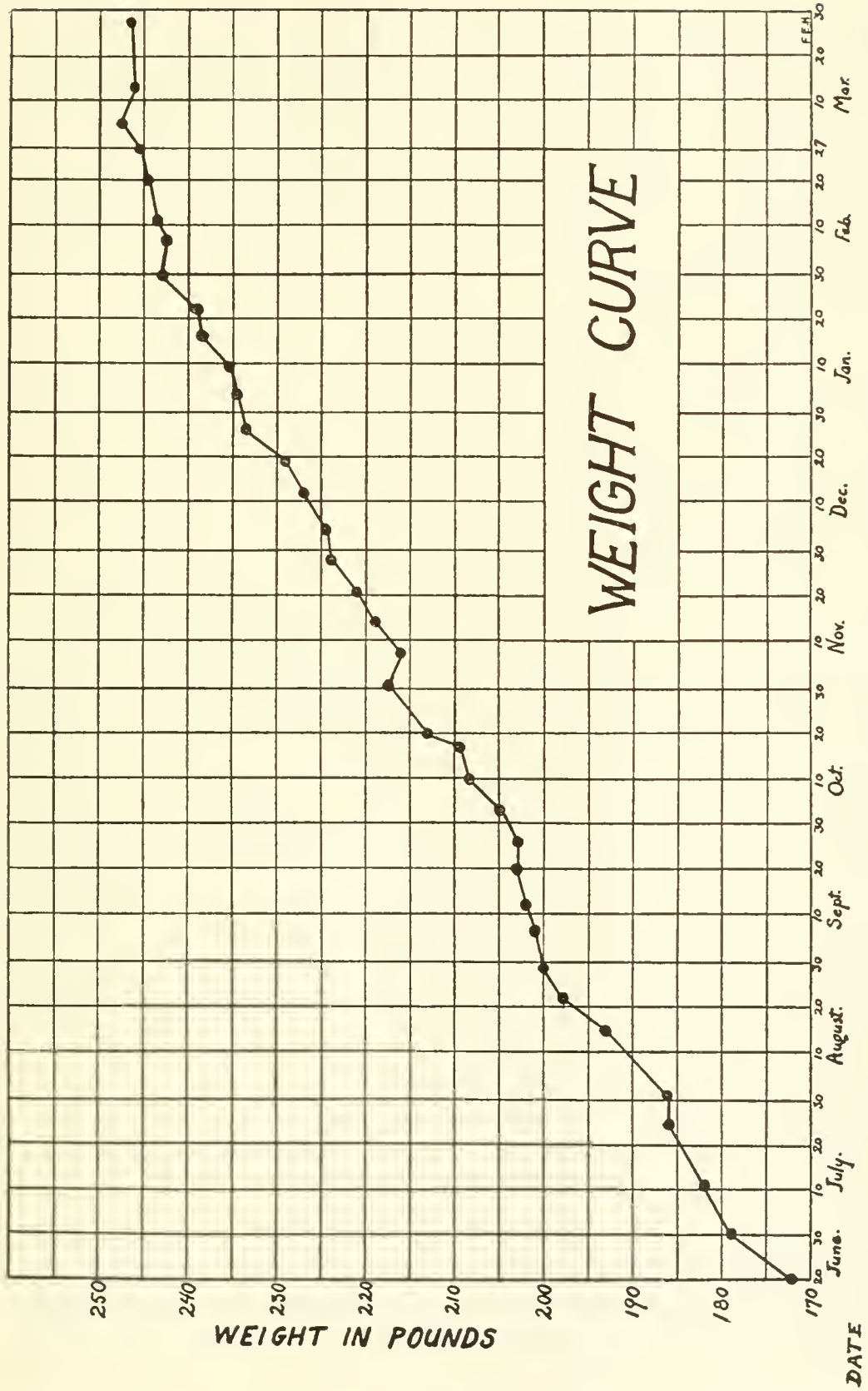

FIG. 11. WEIGHT CURVE ILLUSTRATING REGULAR INCREASE OF WEIGHT IN PATIENT RECOVERING FROM A FOURTH ATTACK OF DEPRESSION

Influence of weight on Pignet Index III

according to Henckel, he had changed from the athletic type to the pyknic type, merely by gaining weight (see fig. 10).

3. Patient no. 10. Weight on June 20, 78.2 kgm. His Pignet Index was −2.9. He gained weight very regularly so that by March 27 his weight was 112 kgm. His Pignet Index therefore had changed from −2.9 to −36.7, that is to say to a value beyond the range of variations given by Henckel for the Pignet Index. He remains still of the pyknic type, although the index values changed so enormously, because beyond the pyknic Henckel has no other type (fig. 11).

If we assume a patient with stature 164.9 cm., chest circumference 87.0 cm., weight 66.0 kgm., the Pignet Index would be 11.9, i.e., the average value for the athletic type according to Henckel. If this patient gains 17.4 kgm., a possible change of weight in the course of a psychosis, his Pignet Index will become −5.5, or average pyknic. If on the other hand this same patient loses 22.4 kgm., his Pignet Index becomes 34.3 or (according to Henckel) reaches the average value for the asthenic type. Such considerable changes of weight are not at all infrequent in mental disease. However, very much smaller weight changes influence the Pignet Index values to such an extent that (according to Henckel's figures) an individual would be transposed from the boundary of one group to that of another, even though he might not reach the average value for that group.

It seems evident therefore that anthropometric indices which contain weight as a component are not very suitable for exact evaluation of the morphological habitus of mental patients, although they may have proved very useful for classifying the degrees of fitness of recruits. Index valuations containing weight can be used only if the weight curve throughout the psychosis from the onset, not only from admission to the hospital, is recorded.

If attention is drawn here to the fact that weight is a source of error in so-called constitutional indices, it is not implied that weight itself may not be of importance as a constitutional sign. In fact changes of weight are probably one of the most important constitutionally determined physiological reactions. It is a common medical experience that certain people are constitutionally apt to gain weight under conditions when other people do not. In mental diseases it is

sometimes striking how psychopathological reactions which in many ways are similar show different weight curves during the psychosis. Kraepelin reproduces in his text book ((47), p. 1229) two curves of

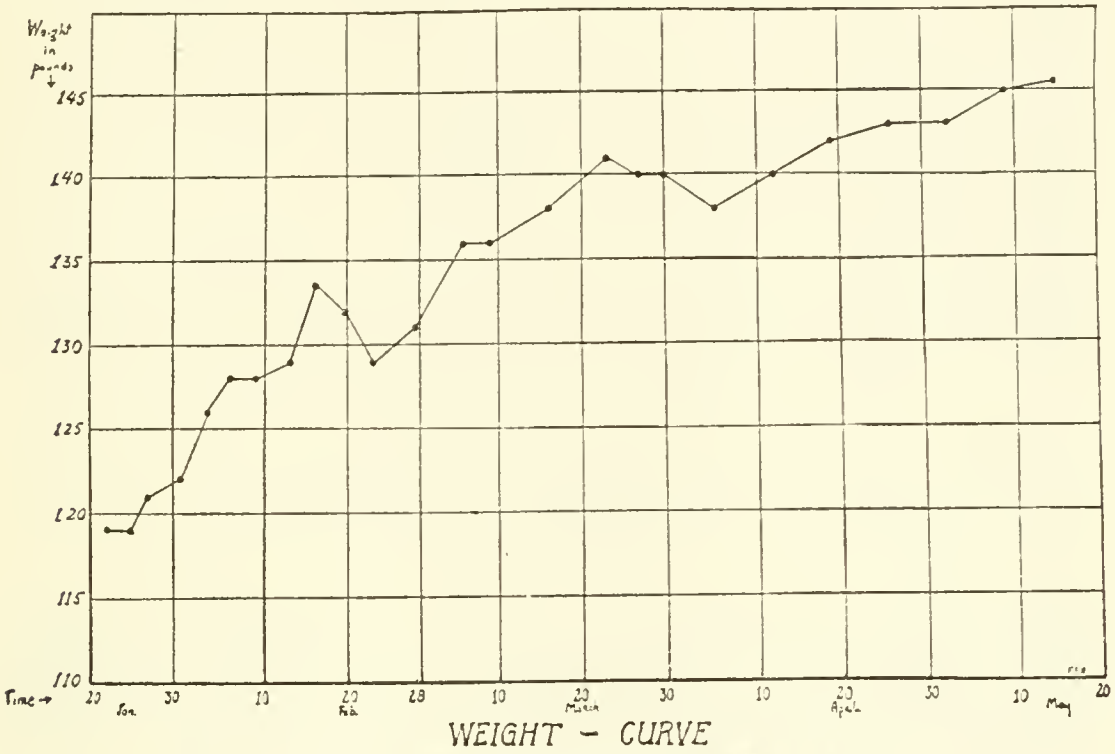

FIG. 12. REGULAR WEIGHT CURVE DURING MANIC ATTACK

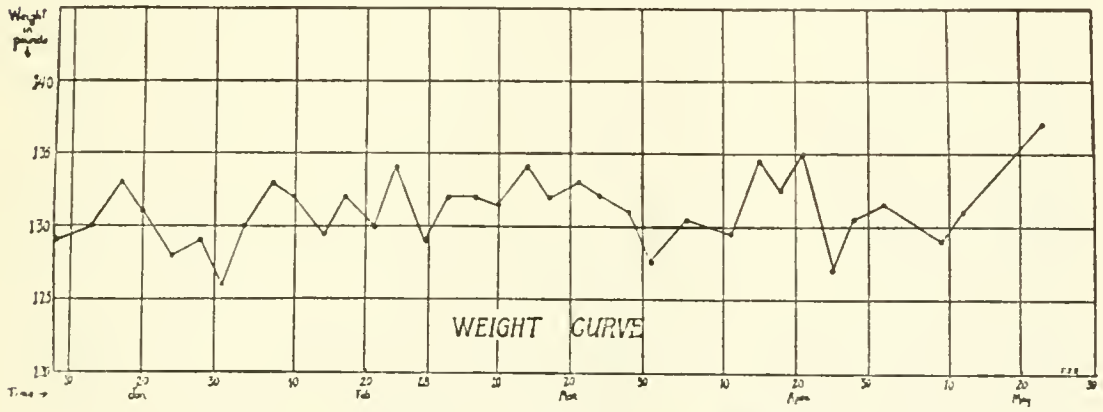

FIG. 13. IRREGULAR WEIGHT CURVE DURING MANIC ATTACK

manic patients—one regular, one very irregular. He assumes for the explanation of the irregular weight curve that the manic attack was composed of many small attacks. Figures 12 and 13 show the weight

curves of two patients with predominantly manic excitement from which both recovered completely. The first curve is fairly regular, the second very irregular. The first patient suffered from a manic excitement with religious grandiose delusions and an episode with auditory hallucination. The second patient suffered from a manic excitement complicated by dysmnesic and certain schizophrenic-like reactions. His sister suffered from a recurrent stuporous reaction with a normal interval of about ten years. The first patient was of pyknoid habitus. The second was of athletic habitus. It is possible that the differences in the weight curve of different mental patients are at least related to the morphological constitution in general. The physiology of the loss and gain of weight in mental diseases is not yet at all clear. From our observation it seems possible to make three factors responsible for weight changes: first, the variation in the actual intake of food; secondly, the often-called endogenic disturbances of metabolism, which according to Reichardt (72) are probably due to a disorder of the central nervous system; thirdly, a constitutional factor which may be correlated in some way with the morphological constitution. In different psychoses these three factors are involved in varying degrees. Reichardt (72) has observed greater endogenic weight changes in schizophrenia than in manic-depressive psychoses (p. 339). An old observation by Nasse (quoted by Rosenfeld (64)) is of interest in this connection. He found that patients who had suffered from previous psychotic attacks tended to show less change of weight during the psychosis. It seemed to him that a constitutional disposition to mental disease may have a certain connection with only slight changes of body weight. In this form Nasse's remark is probably not correct, as is shown by the weight curve of patient no. 10 (fig. 11), who suffered from his fourth recurrent depression each of which was accompanied by considerable loss of weight. Nevertheless there may be a difference in the weight changes of patients with many recurrences and those with only one attack (according to Pollock (69) more than 50 per cent), on the basis that recurrence of attacks may be regarded as a constitutionally determined sign correlated more frequently than other manic-depressive reactions with a characteristic bodily habitus.

A NEW ANTHROPOMETRIC INDEX; CHARACTERISTIC "TYPES" AT ENDS OF FREQUENCY CURVE

Starting primarily from the morphological conceptions of the Italian school (de Giovanni, Viola) an attempt was made to construct an anthropometric index which would indicate in toto the variations in relationship between trunk cavity and limb length. The greatest difficulty is to obtain measurements indicating the morphological development of the trunk cavity from skeletal points alone and without reference to factors influenced by weight. The skeletal part of the thorax and the trunk height were used as the skeletal substratum of the three-dimensional volume of the trunk. The trunk volume was therefore expressed as the product of three measurements:

1. Transverse chest diameter
2. Sagittal chest diameter
3. Trunk height

In order to obtain the relation to limb length the product of these three measurements was combined with the length of the leg. The measurement of the leg length is an indication of the limb length because the ratio of arm to leg length is one of the most constant of human proportional relationships. The formula of the index is therefore:

$$\text{Index} = \frac{\text{Leg length} \times 10^3}{\text{Transverse chest diameter} \times \text{sagittal chest diameter} \times \text{trunk height}} \times 100$$

All measurements are expressed in centimeters. The leg length is multiplied by 10^3 to bring it from the linear to the cubic dimension and in this way relate it mathematically to the cubic denominator, composed of the product of three linear measurements. The quotient is multiplied by one hundred in order that the result or index may be expressed without decimals. In figure 14 the sixty-five cases are represented in a frequency distribution curve. The abscissa gives the values of the index, each number corresponding to the values from that number to the next higher one; for example in the ordinate column erected over two hundred and forty are all cases with index values from two hundred and forty to two hundred and fifty-five. The

individual cases are plotted on the ordinate. The body types are specified in the key. It will be seen that *the values of the index are in definite relation to the body types obtained from observation.* On the

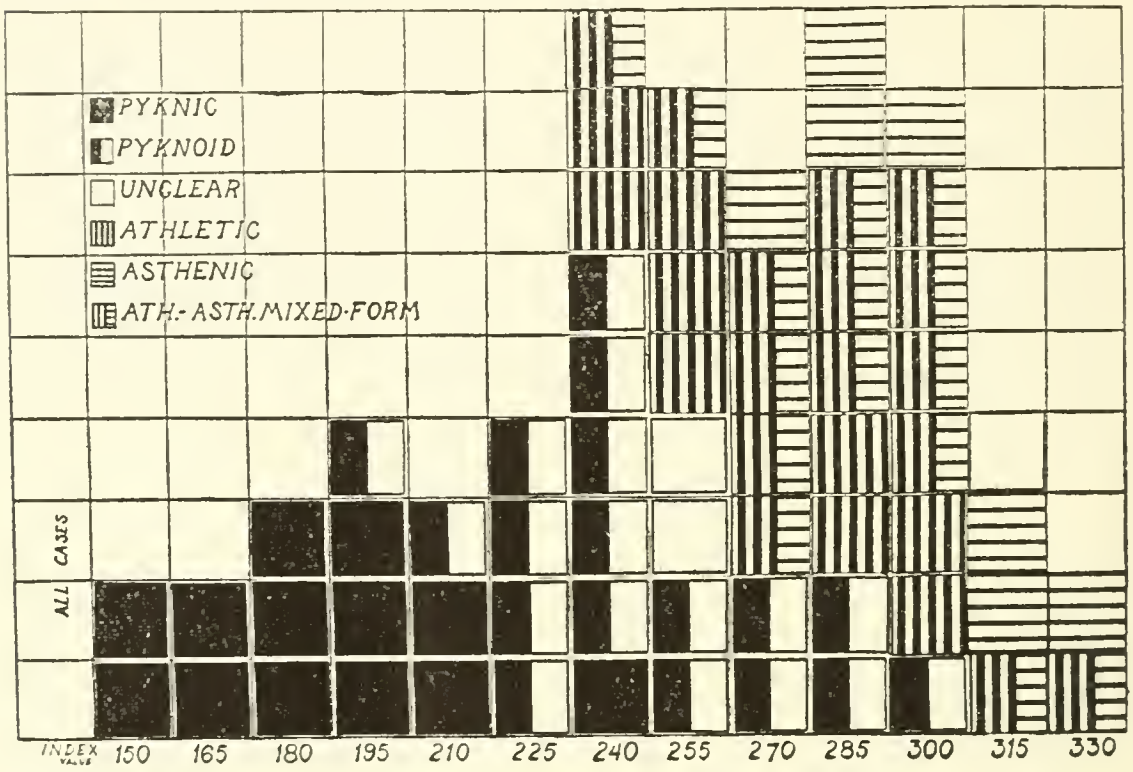

FIG. 14. FREQUENCY DISTRIBUTION OF 65 PATIENTS ACCORDING TO THEIR INDEX VALUES, SHOWING THE CORRESPONDENCE BETWEEN INDEX VALUES AND OBSERVATION TYPES

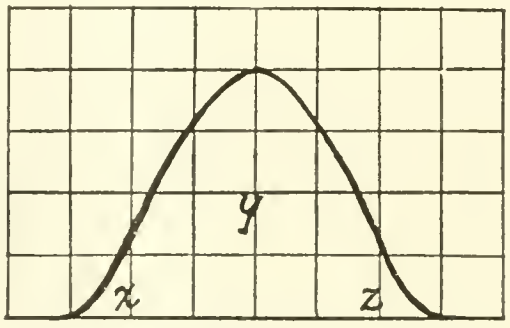

FIG. 15. A NORMAL FREQUENCY CURVE

left side, where the lowest index values occur, are the pyknic types. At the right side, where the highest index values occur, are the asthenic types. There is no overlapping of asthenic and pyknic types. They are separated by the group of cases with index values ranging from

two hundred and fifty-five to two hundred and seventy, in which there are neither pyknics nor asthenics. In the middle are the pyknoid and athletic types, and the asthenic-athletic mixed forms. The pyknoid types tend to the lower, the athletic to the higher, index values. The asthenic-athletic mixed forms tend to higher values of the index than the athletic types. The three cases which were unclear as to classification with regard to body types from the description alone are scattered from the middle to the right side. None of them occur in the pyknic group. Pyknic and non-pyknic forms are clearly differentiated.

The chart indicates *a gradual transition from lower values to higher values of the different clear and mixed types represented.* This seems to be a significant finding because it is established on anthropometric measurements alone without deduction from general types. The chart seems to indicate also that with a sufficiently large number of cases the distribution of body forms would present a normal frequency curve. This distribution would be represented on a curve like that in figure 15. The most clearly differentiated forms would be at *X* and *Z*, the less characteristic forms, intermediate types and gradations in the portion of the curve designated *Y*. This distribution would explain why analysis of varied anthropometric data in search of indications of the existence of out-standing types (indicated by frequency distributions giving bi- or multi-modal frequency distribution curves) proved unsuccessful.

The general distribution according to values of the index seems to coincide in principle with the results of investigations by Viola and also by Bauer, who used the descriptive types of Sigaud. Viola (6) found among four hundred male individuals of Northern Italy much overlapping between the megalosplanchnic and normosplanchnic habitus on the one hand and between the normo- and microsplanchnic on the other (according to his classification). Bauer (6) who recorded the types of Sigaud in two thousand cases at the Vienna Dispensary also found a preponderance of so-called mixed forms. From our material the smaller number of well-defined morphological types in comparison with the others seems well established.

It is possible—even probable—that if a sufficiently large number of cases were collected and classified according to the index, the resulting curve would be asymmetric, or a so-called skew curve, with the peak

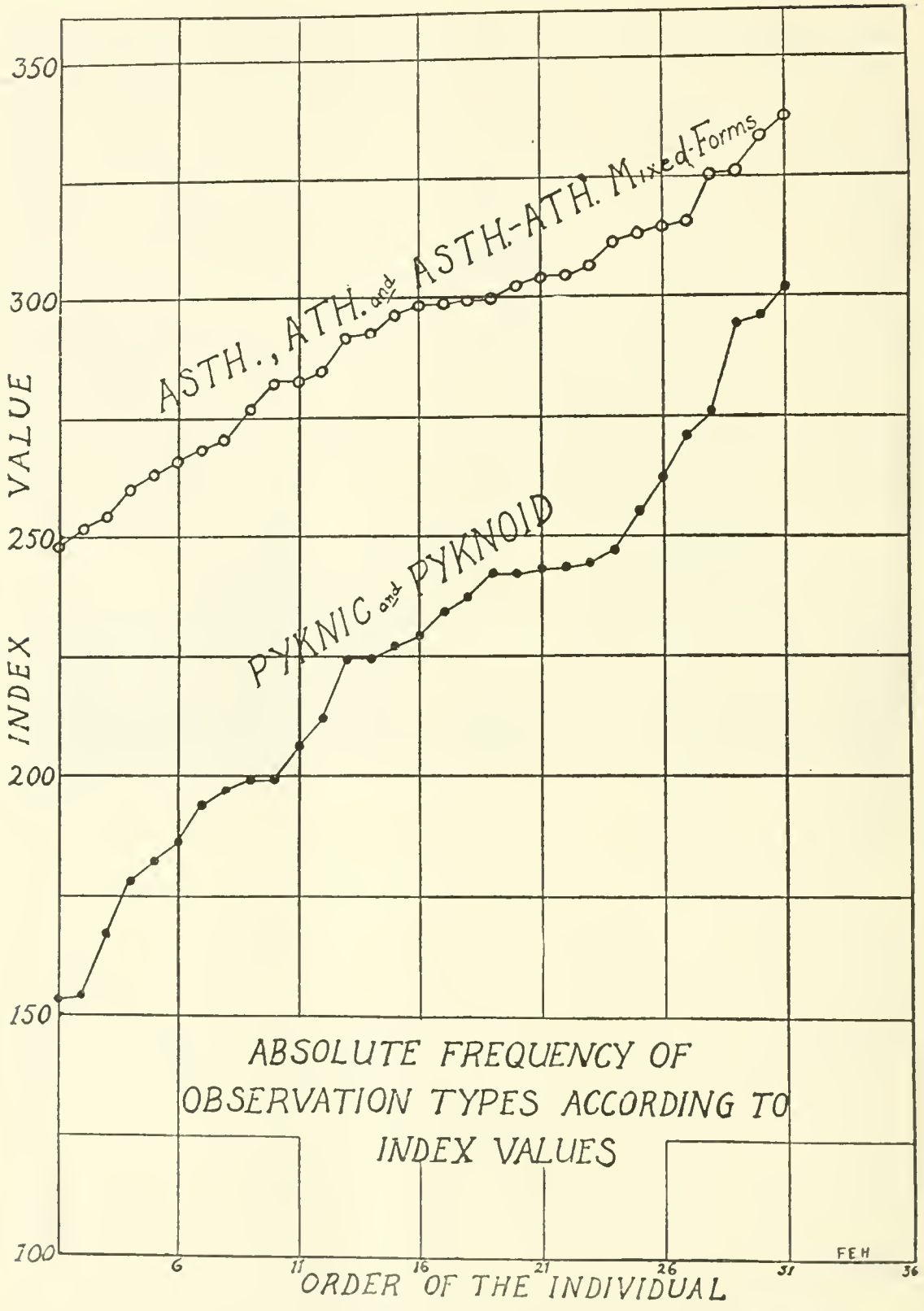

FIG. 16. ABSOLUTE FREQUENCY CURVES OF OBSERVATION TYPES ACCORDING TO INDEX VALUES, ILLUSTRATING THE DIFFERENCE BETWEEN THE PYKNIC AND PYKNOID AND THE ASTHENIC-ATHLETIC FORMS OF BODILY HABITUS

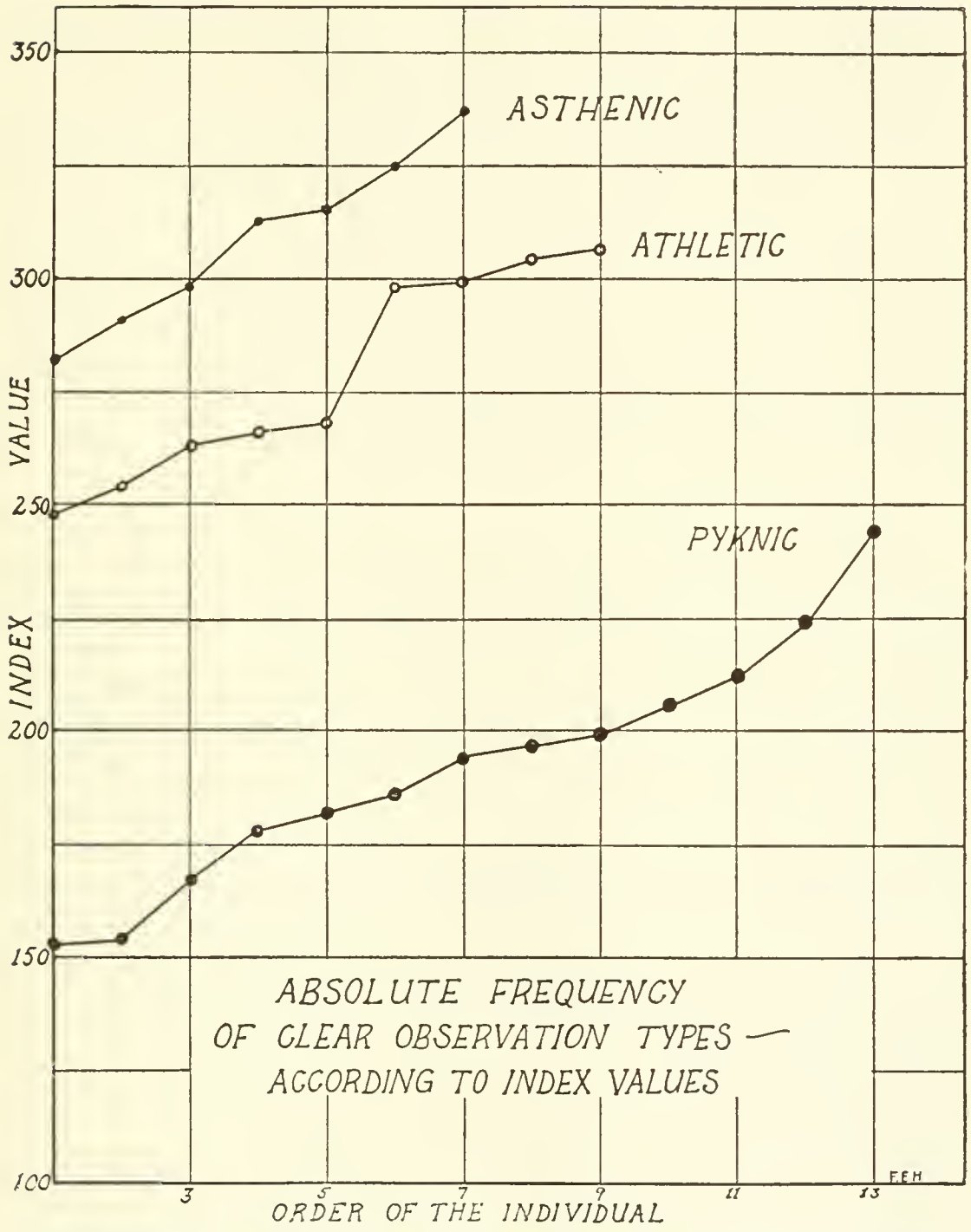

FIG. 17. ABSOLUTE FREQUENCY CURVES OF CLEAR OBSERVATION TYPES ILLUSTRATING DIFFERENCES IN INDEX VALUES BETWEEN THE ASTHENIC, ATHLETIC AND PYKNIC TYPES

not in the middle but toward either side. This study is not intended for these anthropological problems, but only for clinical ones, and the material is also too small to allow definite percentage conclusions.

Moreover it deals only with a selected group of the population, namely individuals afflicted with mental disease, while the proportions of the general population are not accurately known.

In figure 16 the asthenic, athletic and asthenic-athletic mixed forms are contrasted graphically with the pyknic and pyknoid forms in ogive curves[29] according to their index values. The two curves are entirely separate and do not show any tendency to cross. There is a considerable interval between any two contrasted points along their course. This can be taken as an indication that the material does not represent one homogeneous type, but that on the contrary at least two types are present. If the two ogive curves represented cases of a homogeneous type a crossing or intermingling of the two curves would be expected.

In figure 17 the absolute frequency of those types which appear from observation as more or less clear is represented according to their index values. The pyknic type forms the lowest curve, the athletic type the middle, and the asthenic the highest curve. This chart indicates that the anthropometric values of the index separate these three types, the distinction between the asthenic and pyknic type being very considerable and beyond doubt. The curve of the athletic type lies between the two, but definitely nearer the asthenic, indicating that with regard to trunk volume and limb proportions the athletic type is nearer the asthenic than the pyknic type. Thus the anthropometric values of the index permit an exact statement which is in conformity with the more general impression received from observation. It is improbable that the distribution on this curve is due to the small number of cases.

The average index value and the range of variation for each morphological group are as follows:

	INDEX	RANGE OF VARIATION
Asthenic	309	(282–337)
Athletic	278	(248–306)
Pyknic	192	(153–244)

[29] The ogive curve, so-called by Galton who devised it, has been chosen as the graphic method of representing the absolute frequency distribution. According to this method the measurement (in this case the index) is plotted on the ordinate axis, the absolute frequency = order of the individual, along the abscissa. So at equal intervals along the abscissal axis an ordinate is erected for each individual, representing his index value.

The difference between the index values of asthenic and pyknic types is therefore not only great (117), but there is also no overlapping between the extreme members of the group. The highest pyknic index is 244; the lowest asthenic index is 282. The athletic group is in an intermediate position between the other two.

ANALYSIS OF THE VARIOUS COMPONENTS OF THE INDEX

Since the index used contains four different components, each of which may be variable, an analysis of the variation of these components is necessary. The following table gives the average values with the range of variation for each component of the index. The measurements are given in centimeters.

MEASUREMENT	ASTHENIC	ATHLETIC	PYKNIC
Trunk height	49.7(53.1– 62.7)	57.0(52.0–60.8)	58.9(51.7–64.9)
Leg length	85.6(78.3–101.6*)	88.2(75.2–92.2)	85.2(77.5–92.5)
$\frac{\text{Trunk height}}{\text{Leg length}}$ index†	66.7(62.5– 69.2)	64.9(60.1–71.1)	68.9(61.9–79.0)
Transverse chest diameter	25.6(24.3– 28.7)	28.0(26.0–29.8)	31.3(28.7–34.6)
Sagittal chest diameter	19.1(18.1– 20.7)	19.9(17.8–23.5)	24.4(22.0–29.0)

* Case no. 20; see below.

† Since the absolute measurements are much less significant the trunk length expressed in percentage of the leg length is added to the table giving the measurements contained in the index.

It will be seen that the trunk height shows considerable difference. The absolute leg length shows practically no difference between asthenics and pyknics, although under the asthenics is included case no. 20, a case with dysplastic body form, his legs being disproportionately long. The trunk height-leg length index shows a 2 per cent difference between each group. A considerable difference is also present in the chest diameters. It seems of significance that those components of the index which show the most marked differences, namely, trunk height and chest diameter, show an increase in value from asthenic to athletic to pyknic, the athletic being in the middle just as in fig. 17.

AVERAGES OF SOME OTHER ANTHROPOLOGICAL DATA

Our interest being primarily a clinical rather than an anthropological one, we are less concerned with anthropological details. Nevertheless, some other measurements seem to add not insignificant characteristics, and may be of interest for a morphogenetic interpretation of these types. The following averages and ranges of variation are therefore given:[30]

	ASTHENIC	ATHLETIC	PYKNIC
1. Body surface (in sq. m.)	1.65(1.49–1.97*)	1.71(1.41–1.90)	1.96(1.72–2.29)
2. Subcostal angle (in degrees)	55.0(50.0–60.0)	64.5(50–75)	73.0(55–90)
3. Head circumference (in cm.)	56.4(54.5–58.0)	56.3(54.2–59.0)	57.5(53.4–60.5)
4. Cephalic length-breadth index	76.9(69.9–86.1)	78.6(75.1–85.2)	79.4(77.5–80.9)
5. Cephalic height-length index	68.7(63.4–74.6)	71.1(65.7–78.2)	70.1(65.6–74.9)
6. Cephalic height-breadth index	88.3(81.4–100.0)	90.6(81.2–99.3)	88.5(78.8–93.6)
7. Bi-iliac diameter (in cm.)	28.0(25.1–31.2)	29.1(26.7–34.0)	32.2(28.9–36.5)
8. Bi-acromial diameter (in cm.)	36.2(33.3–39.4)	39.1(35.5–41.9)	39.3(33.9–43.0)
9. Bi-acromial diameter/bi-iliac diameter index	1.31(1.17–1.41)	1.34(1.23–1.41)	1.23(1.07–1.35)

* Case No. 20 (dysplastic). The next figure is 1.69.

It will be seen that there is a marked difference between the pyknic and asthenic body types with regard to body surface and to subcostal angle, the athletic type taking an intermediate position in relation to the measurements of the other two body forms. The head measurements do not show any marked distinction. Measurements of considerable interest because of the relative and absolute proportions they indicate, are those of the bi-iliac and bi-acromial diameters. (The bi-iliac diameter is taken at the outer border of the iliac crests, according to Martin's technique.) The preceding figures show that the absolute measurements of the pyknic are greatest. However, con-

[30] These figures, based on a relatively small number of cases, can have significance only as indications of the proportions to which the types tend, and cannot be expected to adequately delineate the types.

sideration of the relationship between these two measurements in each type group shows that the pyknic habitus has least difference between shoulder width and pelvic breadth (considering these measurements as indicators of these two dimensions), while the asthenic comes next, and the athletic has the greatest difference between these two dimensions. It will be recalled that the pyknic type was described as having a barrel-like trunk, largest in the middle dimensions, and returning at the pelvis to proportions comparable with the shoulder breadth. It is also of interest to note that the impression of the broad-shouldered trapezoid-like trunk of the athletic is upheld by these figures. The figures for the asthenic type indicate a more slender type, especially as contrasted with the stocky pyknic form.

Considering the anthropological significance of data concerning hair and eye color, the fact that analysis of these data in the types of habitus considered here yielded no results in any way significant, is not without interest. Regarding complexion the generalization can be made from our material that as a rule the pyknic type has a fresher and more ruddy color, and a clearer and more smooth skin, than occurs in the other two types.

FACTORS INFLUENCING THE MORPHOLOGICAL HABITUS; THE RACE FACTOR; AGE AND GROWTH; ENVIRONMENTAL INFLUENCES (OCCUPATION)

A number of factors influencing bodily habitus have to be considered. That racial distribution is not a causal factor for these morphological types as has been claimed by Stern-Piper (83)[31] is apparent from our material, which is an example of the racially heterogenous American population. In most cases both maternal and paternal extraction could be obtained. Our patients represent parental extraction of the following character: Scotch, Irish, English, Dutch, French, Norwegian, North German, South German, Welsh, Swedish, Canadian, Portuguese, Russian, Italian, Polish and Czecho-Slovakian. It is possible, in fact very likely, that different races show a different distribution of these body types. Stockard (85) believes that the English people of the upper class show more often the linear type,

[31] Compare also von Rohden (74) who expresses the opposite view.

Germans of the upper class more often the lateral type. Whether they occur among all races is not yet established.

Much more important is the age and growth factor. The original assumption of Sigaud that his morphological types are unchangeable during life had to be given up. Viola believes that the longi- and brachy-types are unchangeable and independent of changes with age. Zweig (95) found that although a definite tendency to the four Sigaud types is already apparent in youth, they are not so unchangeable in the course of life as Sigaud had claimed. Especially the digestive habitus shows progressive increase with age. Bauer (6), p. 1086, also found age displacement of the Sigaud types. Brugsch (11) found that the narrow chested type develops to a normal chested type in one-third of all cases from the age of twenty-five to thirty-five. The normal chested type can develop with advancing age to a wide chested type (pathological emphysema type). Florschütz (21) found in a large statistical material that asthenic individuals do not lose that type, but that with advancing age the type becomes even more apparent. Mills (59) distinguishes dominant types (hypersthenic and asthenic), major types (hypersthenic, sthenic, hyposthenic and asthenic), and six sub-types. According to his observations on over one thousand individuals, bodily changes may lead to a reclassification of an individual with regard to a different sub-type, but no changes occur indicating a reclassification as to major type.

The environmental influences on morphological habitus, which of course become apparent at different ages, are considered in the researches of Verschner on the anthropological characteristics of single-ovum twins (89). According to his findings, which, however, are not final and not in agreement with some other observations, the following characteristics are little affected by environmental influences in the widest sense: stature, length of extremities—absolute and relative to stature, ears, nose, eyes. Much influenced are the following: length, breadth, depth and circumference of trunk, excluding shoulder and thorax breadth, body weight, length, breadth and length-breadth index of skull. The difference between twins does not essentially increase with further years after the end of the age of growth.

In our material the age distribution is as follows:[32]

	AVERAGE	RANGE OF VARIATION
	years	
According to types:		
13 pyknics	45.1	(24–59)
7 asthenics	29.0	(16–52)
9 athletics	28.7	(20–51)
According to diagnosis:		
19 predominantly affective	36.2	(18–65)
25 predominantly schizophrenic	28.6	(18–39)
21 other cases	38.8	(22–56)

The pyknic type is more frequent with advancing age. Bauer found this also for the digestive type of Sigaud. For clinical correlations this is of special importance since manic-depressive psychoses occur more frequently in middle age. Möllenhoff (60) has pointed out the importance of the age factor for these correlations. He found the asthenic type more frequently among his young patients and the pyknic type more frequently with advancing age.

From the anthropometric analysis the conclusion was also reached that the age factor is very important and that there is a far-reaching age displacement of body types. That age on the other hand should be the only factor responsible seems extremely unlikely. There are cases of definite pyknic habitus with low index at an early age, and of pronounced asthenic habitus with high index at an advanced age. Moreover, as Kretschmer (45) has pointed out, there are details of observation which according to clinical experience change very little with age, for example the form of the hand and head, and the color of the skin.

The ratio of the body length below the symphysis to that above the symphysis shows very marked changes with growth. The line dividing the stature in half in the infant lies considerably above the symphysis. In adult age the length of the upper part of the body (above the symphysis) becomes greater than the height from the symphysis to the floor. According to studies of Fischer and Hoffmann (20) on three hundred and sixty healthy soldiers, this constant predomi-

[32] See also page 430.

nance of the length of the upper part of the body is reached in one hundred per cent of the individuals by the twenty-ninth year. In our material the exact ratio of the upper to the lower part of the body is variable (when considered in reference to age). At the age of twenty-nine it may be assumed that at least the maximum growth of the thorax is completed. For these reasons separate correlations between index values and psychiatric diagnosis were made with the exclusion of all patients under twenty-nine.

For dysplastic features the age factor is less significant. The pubic hair, for example, according to the painstaking statistical studies of Scheidt (77) shows the full virile growth about the eighteenth year. The distribution type is finally established by the end of the second decade. The average age of patients in the dysplastic group is thirty, the range of variation 16 to 51.

The influence of occupation seems on the whole of little significance for the essential morphological characteristics. With regard to occupation our material is divided according to the old and somewhat arbitrary classification of Gould, which nevertheless is sufficiently useful:

I. Those who work with the head
II. Those who work mainly with the arms
III. Those who work with arms and legs (sailors)

Our material falls into the following classes:

According to diagnosis

All cases	I	35
	II	27
	III	3
Manic-depressive, I		19 (all)
Schizophrenic	I	9
	II	14
	III	2
Organic	I	2
	II	7
	III	1
Psychopathic personalities and psychoneuroses	I	5
	II	6
	III	0

According to types

13 pyknic	I	5
	II	7
	III	1
9 athletic	I	4
	II	5
	III	0
7 asthenic	I	5
	II	1
	III	1

According to source

	Henry Phipps Psychiatric Clinic	*Spring Grove State Hospital*
Manic-depressive	18	1
Schizophrenic	11	14
Organic	7	3
Psychopathic personalities and psychoneuroses	9	2
	45	20

It would seem that the affective cases belong more to the group of mental workers. This agrees with Stern (82) and Möllenhoff (60), but is in our material partly explained by the fact that the patients come from two sources drawing patients to some extent from different social strata.

CORRELATION OF PSYCHOSES AND PREPSYCHOTIC PERSONALITIES WITH INDEX VALUES AND BODY TYPES FROM OBSERVATION

It is possible then to distinguish from the morphological aspect different groups in this series of sixty-five cases. For clinical correlation we are now in a position to dispense with the body types obtained deductively from the general impression and can substitute the inductive method of comparing the anthropometric values of the index with psychiatric diagnostic groups, without knowledge of what the general type impression from observation would be.

In table 2 all sixty-five cases are represented with a serial number, initials, order in the four diagnostic groups, special psychiatric diagnosis, body type from observation, index value, and incidence of dysplastic features.

It may be assumed from the distribution of the cases according to index values (see fig. 14) that the extreme values of the index may be regarded as characteristic recurring body forms which impress one as morphological body types. If the whole range of index values in the first two psychiatric diagnostic groups is divided into four equal parts, the opportunity is created to determine what psychopathological reactions have an affinity to the index values which are very low (lowest quarter) and those which are very high (the highest quarter). In the range of the lowest quarter of the index values there are only three patients with the following index values: 154, 167, 182. All these three patients belong clearly to the manic-depressive reaction type. The first one (no. 10) was admitted in his fourth depressive attack. The second one (no. 16) was admitted in his second depressive attack. The third patient (no. 6) suffered from a depressive reaction. In the highest quarter of the range of index values there are, leaving out those with dysplastic features, twelve patients. Of these twelve patients seven belong to the schizophrenic reaction type (nos. 25, 39, 21, 43, 35, 22, 23). Two belong to the predominantly affective group but are specified as showing schizophrenic features during the psychosis (nos. 18 and 19). One case (no. 9) occurs in the affective group but is specified as psychoneurotic depression. Only two cases belong to the manic-depressive reaction type without qualification (nos. 3 and 11). Both are very young. This seems to point to the conclusion that the lowest index values occur more among the manic-depressive reaction types and the higher index values among the schizophrenic individuals. If patients with dysplastic features are included there is added to the lower fourth one patient (no. 41) with the diagnosis schizophrenia and to the upper fourth of the range of index values six patients with dysplastic features (nos. 27, 31, 8, 36, 28), all of whom belong to the schizophrenic reaction type with the exception of one (no. 8) who occurs in the predominantly affective group but is specified as showing schizophrenic-like admixtures.

If the index values are now split into thirds it can be determined what psychopathological reaction types occur in the lower third and which in the upper third, including in both groups individuals showing dysplastic features. In the lower third of index values there are six

patients, in the upper third twenty-one patients (including only the first two psychiatric diagnostic groups). Of the group of six patients with low index values, four patients belong to the manic-depressive reaction type (nos. 10, 16, 6, 7). The last patient is in this connection of particular significance. He suffered for seventeen years from a chronic manic condition from which he recovered with good insight. We had the opportunity of seeing this patient during the psychosis, during recovery, and in the normal period after his recovery, and had then occasion to observe him again in his second attack, which was clearly of a manic nature and from which he also recovered completely after about four months.[33] Of the two other patients in the lower third of the index values, one (no. 41) showing dysplastic features, belongs to the schizophrenic reaction type; the other one (no. 24) occurs in the schizophrenic group, but is qualified as an atypical case with anxiety features. It is evident again that among the lower index values the affective psychoses are predominant.

Of the twenty-one patients occurring in the upper third of the index values there are fourteen with schizophrenic reaction type (nos. 30, 38, 25, 39, 27, 21, 43, 35, 22, 20, 23, 31, 36, 28). Three cases occur in the predominantly affective group, but are specified as showing schizophrenic features (nos. 18, 8, 19). One case (no. 9) in the affective group presents psychoneurotic symptoms. Only three patients occur in the manic-depressive group without qualification (nos. 17, 3, 11), two of these patients being the two youngest individuals of the manic-depressive group. The predominance of schizophrenic reactions in this group with higher values of the index is apparent. It is also significant that those three cases in the affective group are included from the morphological aspect in the group with higher index values, which from psychiatric diagnostic principles alone had been previously specified as being somewhat atypical in the affective group because they show also schizophrenic-like reactions. It has to be taken into account that the psychiatric diagnoses were put into their final form before the calculation of the index values was completed.

[33] This case will be presented in more detail in a later study.

If only the more or less clear manic-depressive and schizophrenic cases are selected the following index averages are obtained.

	AVERAGE INDEX	RANGE OF VARIATION
All clear manic-depressive cases (11 cases)	233.2	(154 –315)
All clear schizophrenic cases (23 cases)	280.7	(178*–337)

* Patient no. 41 with dysplastic features; the next higher index value is 229.

Since, however, the age factor as we have seen is of importance and since the manic-depressive patients usually have a considerably higher average age than the schizophrenic patients this has to be taken into account for the psychiatric correlations.

	AVERAGE AGE	RANGE OF VARIATION
	years	
Clear manic-depressive cases	39.3	(18–65)
Clear schizophrenic cases	28.7	(16–39)

If only those cases above and including twenty-nine years are selected (see discussion on page 425) the following index averages are obtained.

	AVERAGE INDEX	RANGE OF VARIATION
Clear manic-depressive cases (6 cases)	199.1	(154 –262)
Clear schizophrenic cases (12 cases)	260.3	(178*–315)

* Patient no. 41 with dysplastic features; the next higher index is 229.

Correlation of all clear manic-depressive and schizophrenic diagnoses with morphological types from observation (including patients with dysplastic features) gives the following percentages:

In clear manic-depressive cases, all ages

	per cent
Pyknic	45.5
Pyknoid	36.4
Asthenic	0
Athletic	9.0
Unclear	9.0

In clear schizophrenic cases, all ages

Pyknic	4.3	
Pyknoid	13.0	
Asthenic	17.4	
Athletic	26.1	78.3
Asthenic-athletic mixed	34.8	
Unclear	4.3	

In clear manic-depressive cases 29 years and over

Pyknic	66.6	
Pyknoid	33.3	
Asthenic	0	
Athletic	0	

In clear schizophrenic cases 29 years and over

Pyknic	8.3	
Pyknoid	25.0	
Asthenic	16.7	
Athletic	16.7	58.4
Asthenic-athletic mixed	25.0	
Unclear	8.3	

The different diagnostic groups are too small to allow a differentiation according to special types of schizophrenic or affective reaction. However, from the schizophrenic group may be selected all those cases with catatonic phases. Three patients in this group show catatonic symptoms (patients nos. 22, 23, 27). All three have indices in the upper fourth of the range of index values (indices 306, 314, 298). Two patients in the schizophrenic group show simple deterioration (nos. 28 and 43). Their index values are above 300 (302, 337). Individual correlations of each case may be seen on table 2.

Independent of Kretschmer's researches, Beringer and Düser (8) showed the frequent incidence of eunuchoid and feministic morphological traits in schizophrenic patients. Gibbs (23) found feminine distribution of pubic hair in 13 per cent of schizophrenic patients admitted between sixteen and twenty, which was still present in 13.4 per cent after they were twenty-one or over; but this characteristic was present in only 2.6 per cent of those patients admitted between twenty-one and forty.

Twelve patients of the group of sixty-five considered in this study have dysplastic features. Eleven of these showed among other dys-

plastic characteristics, feminine distribution of pubic hair. Figure 18 shows the relation of the dysplastic group of patients to the psychiatric diagnoses of all sixty-five cases. It will be seen that in the first group, consisting of predominantly affective reaction types, there is only one case with feminine distribution of pubic hair (case no. 8). This patient suffered from a psychosis which was diagnosed as predominantly manic-depressive with the schizophrenic features specified on table 2. In the second group, consisting of predominantly schizophrenic patients, are nine cases with dysplastic pubic hair. These nine cases are scattered irregularly throughout the group. Among the psychopathic personalities and psychoneuroses there is no dysplastic

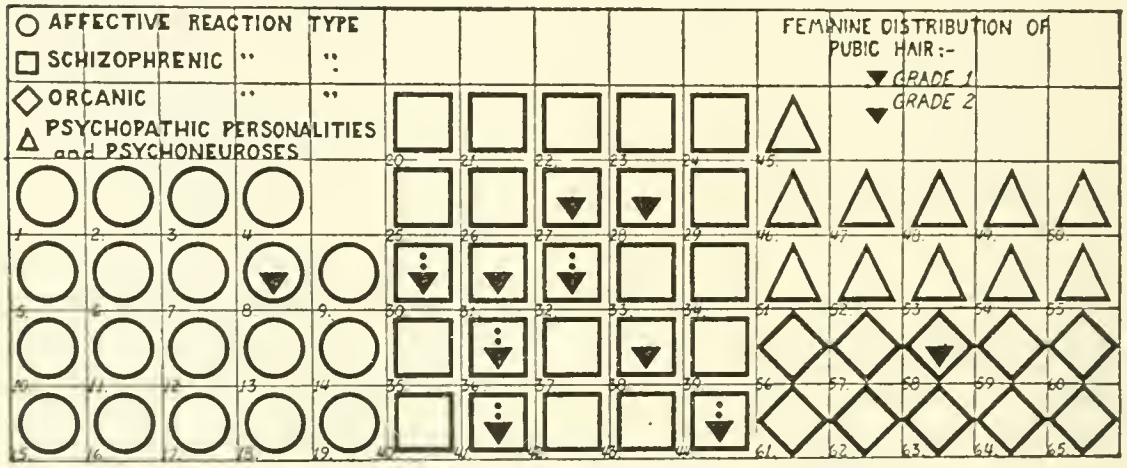

FIG. 18. INCIDENCE OF DYSPLASTIC FEATURES IN THE DIFFERENT DIAGNOSTIC GROUPS ILLUSTRATED BY THE OCCURRENCE OF FEMININE TYPE OF CRINES AMONG 65 PATIENTS

case. One such patient occurs in the organic reaction type group. This patient (no. 58) was a case of dementia paralytica with the somewhat unusual feature that four years before admission to the hospital he suffered from an ill-defined depressive episode. The twelfth patient of the group with dysplastic features, but with masculine crines, also suffered from a schizophrenic psychosis (no. 20). The affinity of the schizophrenic reaction type to this dysplastic group is therefore evident. No conclusions as regards endocrinological factors in schizophrenic reactions can be drawn from this finding, because deficiencies of hair are found not infrequently in the general population. However, the contrast in this respect between the schizophrenic and the manic-depressive groups is striking.

The percentage of the incidence of body types in the general population is not accurately known. While the types of Viola, Sigaud, and Kretschmer, and our group with high and low index values, have a certain similarity, no definite conclusions can be drawn with regard to the incidence of types in the general population from studies which have been done according to only one of these classifications (like that of Viola, see p. 415). For this reason the emphasis of this study had to be necessarily on the contrast between the two psychiatric diagnostic groups—the manic-depressive and the schizophrenic. Apart from the relatively small number of cases in this material, a comparison of the percentages obtained by other authors following Kretschmer (see Kehrer and Kretschmer (39) table on p. 174) is not carried out because these percentages based on the observation of types do not sufficiently take into account the displacement of body types due to age. They all contrast schizophrenic with manic-depressive patients, and it may be inferred that the manic-depressive group shows a higher average age than the schizophrenic cases, if unselected cases are taken. While from this study it seems apparent that, even apart from the age factor, the pyknic and pyknoid forms occur more in manic-depressive patients than in schizophrenic cases, and the athletic, asthenic and dysplastic forms occur more frequently among schizophrenic patients as compared with manic-depressive reaction types, yet it does not seem possible as yet to state exactly how far the age factor is of influence. For example, Kretschmer (45) quotes the statement of von Rohden that in nine hundred and thirty-seven manic-depressive cases of various observers who used his (Kretschmer's) method, the percentage of pyknic body forms is 66 per cent. In our group of clear manic-depressive cases, the percentage of typical pyknic body forms is only 45.5 per cent. If, however, from this group of more or less clear manic-depressive psychoses, the cases with ages under thirty years are taken out, the percentage of pyknic body types rises to 66.6 per cent (see pages 423 and 429). All the percentages of body types in psychoses, based on observation, have therefore to be taken with the greatest caution on account of the age factor. Correlations should start from exact percentages of body types in the general population. Since this is not possible as yet, two psychotic groups are contrasted which unavoidably, at least as far as

patients observed in psychiatric clinics are concerned, are of different age. Kretschmer has not done justice to the importance of the influence of age. He (39) gives three tables in which his schizophrenic and manic-depressive patients are classified in age groups, but they refer only to chest circumference, Pignet Index, and relation of stature to weight. The age factor, therefore, seems at present one of the most urgent problems of investigation with regard to the morphological constitution in psychiatry.

An attempt was made to make independent correlations between morphological type and prepsychotic personality. Popular belief associates broad and slender body-types with all sorts of mental characteristics and habits. Slender people are supposed to be more often cigarette-smokers, broad people more often cigar-smokers. Lovers of alcohol are supposed to be more broad, abstemious people more slender.[34] Broad people are often regarded as more materialistic and sensuous, slender ones as more idealistic and mystical. There are many examples in folk-lore of similar beliefs. That criminals and "bad men" are characterized by certain physical qualities and are often of dysharmonious growth, was a very widespread popular belief until the advent of the modern moving picture era. Homer depicted Thersites with a generally deformed body and misshapen head. Deficiencies in hair growth are also associated with mental characteristics in popular belief, as shown for example in the French proverb: "Salute from afar the beardless man and the bearded woman."

It is not intended here to discuss in detail the many attempts that have been made to distinguish personality types. None of these attempts have been entirely successful. They are all based on simplifications which do not do justice to the complicated dynamic interplay between the personality and the social setting. From the findings of experimental psychology the human personality as a living entity does not emerge. With the "psychodiagnostic test" of Rorschach constitutional differences between individuals can be found, similar to

[34] Cf. the epigram by J. B. Rousseau (1670–1741):

Toujours ces sages hagards,
Maigres, hideux et blafards,
Sont souillés de quelque opprobre;
Et du premier des Césars
L'assassin fut homme sobre.

though not identical with the intraverted and extraverted types as distinguished by Jung. Kretschmer has used the distinction of "schizoid" and "cycloid" personalities. The concept of "schizoid" personality originated from the observation of three classes of individuals: first, the pre-psychotic personality of individuals later suffering from schizophrenic psychoses; secondly, the type of to-some-degree distinctive personalities sometimes found in the ancestry of schizophrenic patients; thirdly, a type of psychopathic personality with habitual psychopathological reactions similar to some extent to certain manifestations of the schizophrenic reaction type. The concept of "cycloid" personality has a similar origin. In this way personality types are postulated in relation to clinical entities. The danger of this procedure lies in the fact that these two very expanded groups are found again and again, because nothing else is looked for (Kahn, (38), p. 48). In the form given to them by Kretschmer they do not constitute a new point of view, but rather the latest ramification of the Kraepelinian system of clinical disease entities. The evidently secondary psychological presentation of these two types is based on two not unitary principles called by Kretschmer the "diathetic" and "psychaesthetic" proportions. There is a great variety of individuals classified under those two types, which makes it doubtful whether one can speak of them as personality "types." Certain individuals described as schizoid personalities can be designated from a clinical point of view with the same justification, and from a therapeutic point of view more profitably, as psychoneuroses. It seems that the indiscriminate use of the term "schizoid" may do a great deal of harm. This term has the double meaning of being in fact closely related to schizophrenia, as in Kahn's theory of the mode of hereditary transmission of schizophrenia and of being in some way psychologically similar to it, as in Bleuler's distinction of schizoid and syntonic.*

In everyday life personalities are judged usually by the affective attitude toward the environment, especially other individuals and the social group. It seems that with regard to the affective attitude it is possible to distinguish among many individuals two directive tendencies, more or less evident throughout the course of life. Some individ-

* The word *syn-tonic* conveys the meaning of a *being tuned together*, a harmony between the individual and his environment which is not the distinctive mark of these individuals who have rather a tendency in their affective life to react together with others, *harmoniously or otherwise.* (See later discussion.)

uals look for satisfaction in contact with others, the emphasis of their affective experiences and reactions being always on the personal environment. Not only do they react emotionally to the experiences of life, entailing coördination or disagreement with others, but they are always fundamentally in affective contact with the personal and social environment. There are other individuals, on the other hand, who find satisfaction in difference, in detachment and isolation from the personal and social environment. The emphasis of their emotional experiences is much less on the other personalities with whom they come in contact during the course of life, and more on the reactions and ramifications of their own mental experiences, both intellectual and imaginative. One may speak of the first group as the syntropic, because the tendency of the affective attitude of individuals included there is to association and contact with the personal and social environment; the second group, with affective attitude characterized by a satisfaction in difference and separation from emotional relationship with other individuals of their environment, one may speak of as idiotropic.[35]

These characteristics are not sufficient to constitute personality types, but are sufficiently distinct to denote antagonistic directive tendencies. The assigning of an individual to the idiotropic or syntropic group must be dependent upon the evidence of more or less habitual reactions in the course of life. Only very rarely do individuals change from one of these extremes to the other. It is not to be understood that syntropic individuals are always well-adapted and in accordance and harmony with the social environment. On the contrary, they are often not well-adapted; but whatever psychological reaction may lead them to social estrangement the fundamental affective tendency to influence the environment, to "get something across," to find satisfaction and consolation in an emotional setting involving other individuals, is often apparent. In the treatment of syntropic individuals with depression one often finds the tendency to suffer not alone, but through and with others.[36] Idiotropic individuals, on the other hand, need not be badly adapted, but may show good social adaptation. This is, however, fundamentally due to lack of

[35] From the Greek word *tropos* meaning turn, turn of mind, affective tendency, and from the Greek *syn-* meaning together with, in company with, and *idios* meaning singular, distinct, separate.

[36] Cf. also cases of double suicide.

emotional contact, rather than by virtue of it. There are certain minor traits in general behavior which are sometimes quite characteristic. Syntropic individuals tend to be "free and easy" in social intercourse, even in actual bodily contacts, for instance walking arm-in-arm with companions; whereas idiotropic individuals are often conscious and self-conscious of the most meaningless and inadvertent physical contacts. While it is not intended to discuss in more detail these differences in affective attitude,[37] an example from the pathological sphere may be given. Delusions of persecution have been distinguished, according to whether the persecution was deserved or undeserved, into delusions which are condemning (belastend) and delusions which are condoning (entlastend). There is, however, this further distinction between delusions of persecution in many cases: idiotropic individuals are often persecuted by individuals or groups which themselves, *according to the patient's point of view and evaluation*, are not socially integrated and conforming, are "different" or outlaws, or entirely individualistic and without any connection with the social scheme: for example, "the Catholics," "the Jews," "the Masons," "the political enemy," "the devil," "the underworld," or, quite indifferently, "men" or "women." Syntropic individuals, on the other hand, often elaborate delusions of persecution where the persecutors are, in the eyes of the patient, the very essence and symbol of social contact and cohesion; for example, "all the inhabitants of the home town," "the police," "all decent women," "the whole family," "all my friends," "the government," "every moral person." Practically the kernel of the group of individuals described by Jung as intravert and extravert, by Kretschmer as schizoid and cycloid, and by Hoch as shut-in personality, is closely related to these idiotropic and syntropic tendencies.

The formulations of the pre-psychotic personalities were made at the same time as the psychiatric diagnoses. It is often difficult to arrive at a conclusion about the pre-psychotic personality of mental patients. Frequently the information given by relatives, for example, is not reliable. It can be observed sometimes that relatives and friends have a distinct tendency, in cases of severe psychoses, to de-

[37] The theoretical and clinical bearing of this train of thought will be discussed in a later study.

scribe the personality as what *in their own evaluation* is as good and "normal" as possible. In mild disorders, on the other hand, they often have the tendency to make it appear to the physician as if the patient had been always somewhat abnormal, and as if there was not much change in him which necessitated his admission to the hospital. This occurrence is not unimportant in a clinical study of the pre-morbid

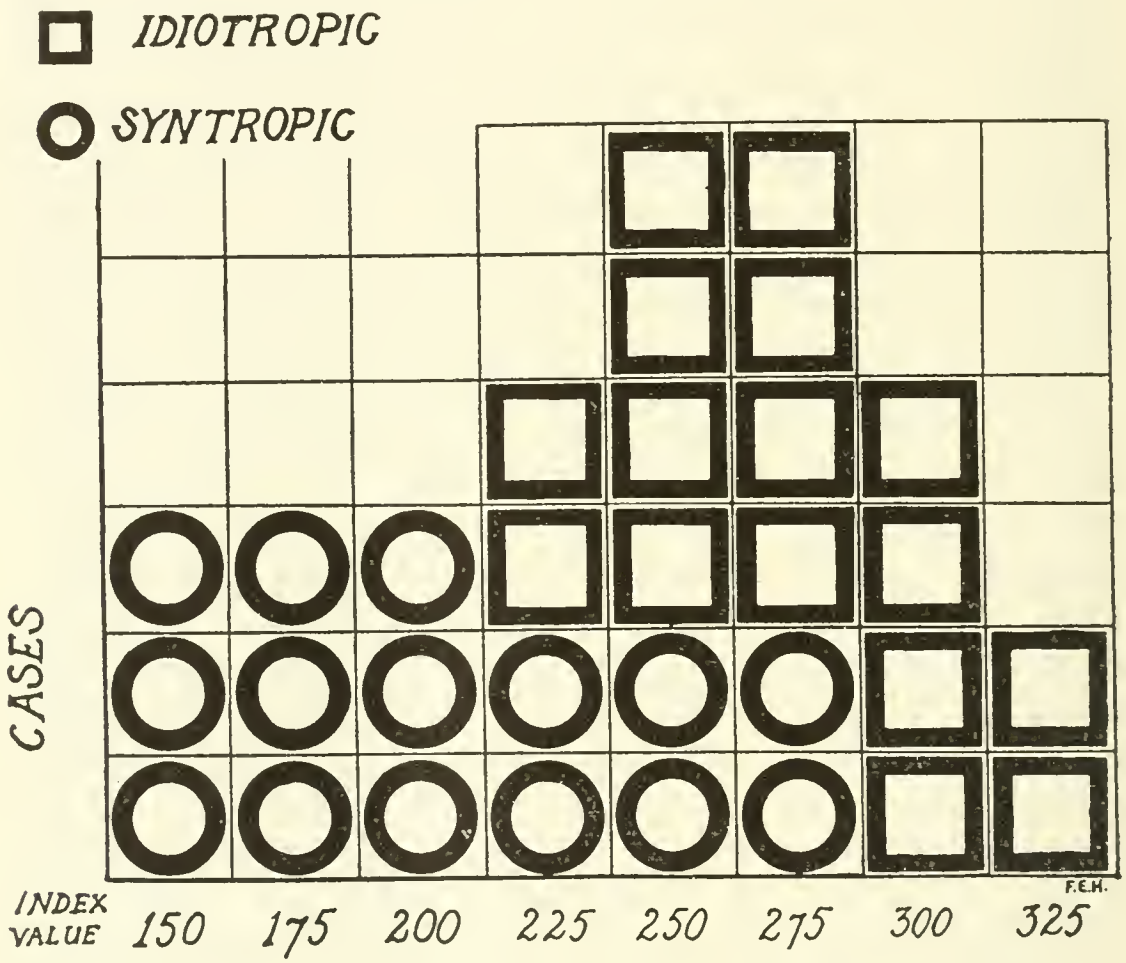

Fig. 19. Relationship between Index Values and Personality Tendencies

personality, and may be spoken of as the phenomenon of distorted personality anamnesis.

The following is an example of a summary of idiotropic tendencies (quoted from 90):

Schizophrenia of predominantly hebephrenic type, with characteristic hyposthenic bodily constitution; school-girl, aged sixteen. Pre-psychotic personality: wilful child; showed little affection toward family, with abrupt

changes from one to another; did not want to be touched or kissed; contrary, reserved, had few contacts with boys or girls; daydreamer. Had crushes on girls and on an older man with whom she had never spoken. Feeling of inferiority; learned well, but did not retain well. Aspired to be either "athletic" or "intellectual;" not as clever as sisters. On basis of inferiority feeling, was selfish, bluffing, gave the appearance of conceit.

Only 31 patients could be assigned to the idiotropic or syntropic groups, either because there was not enough reliable information or because a predominance of idiotropic or syntropic tendencies did not clearly exist.

The correlations between index values and syntropic and idiotropic tendencies can be seen on figure 19.

The average index of the syntropic group is:

217.2 (153–291)

The average index of the idiotropic group is:

289.4 (242–337)

The following are the percentages of body types derived from observation in the syntropic and idiotropic groups:

	per cent	
Syntropic:		
Pyknic	53.4	
Pyknoid	33.3	
Asthenic	6.65	13.3
Athletic	6.65	
Idiotropic:		
Pyknic	0	
Pyknoid	18.7	
Asthenic	18.7	
Athletic	18.7	74.9
Asthenic-athletic mixed	37.5	
Unclear	6.3	

It will be seen that in the syntropic group pyknic forms predominate over the asthenic and athletic types. There are no dysplastic traits occurring in this group. The opposite is true of the idiotropic cases.

The correlations between body-type and personality tendencies seem to be somewhat more pronounced than those with psychoses. This may be due to some extent to the possibility that by this selection

FIG. 20. MR. PICKWICK

From the drawing "A Coaching Dream of Dickens" by Houghton, illustrating the pyknic habitus in a syntropic personality. (Compare description of pyknic habitus, page 394.)

of distinct pre-psychotic personality patterns the more constitutional psychotic reactions were selected. On the other hand it must be realized that from the description of the personality alone, from which the personality diagnoses were made, it can not be said whether or not any of the individuals would later develop a psychosis. That these personality differences exist, and that they may have biological correlations with bodily habitus is very likely. One is reminded of the physical habitus of certain characters in history and fiction, like Mr. Pickwick, the benevolent, genial founder of the Pickwick Club in Dickens' novel (fig. 20), in contrast to the erratic, always isolated Don Quixote, in Cervantes' novel, whose slender body-type is traditional in all pictorial representations.

CONCLUSION; ASPECTS OF THE HUMAN CONSTITUTION

It seems justified to assume that there is a certain correlation between morphological constitution, mental disease and personality. The broader forms of habitus seem to occur more frequently in affective psychoses and syntropic personalities, the more slender body forms in schizophrenic psychoses and idiotropic personalities. Dysplastic traits seem to have an affinity for schizophrenic reactions. A numerical formula for this relationship can not yet be attempted with any degree of exactness. It is tempting to venture upon the genetic interpretation of these types. The dysplastic traits described are undoubtedly similar to certain endocrinological disorders, particularly of the gonads and the pituitary. The morphological and mental changes in cretinistic degeneration and myxoedema, which in the latter through thyroid medication are said to have led to perhaps the most sensational therapeutic successes in the whole field of medicine, are an often-quoted example of endocrine etiology. Only the experiment can show how far this analogy can be carried through. In the psychopathological sphere, the polymorphous picture of the psychopathological conditions due to and influenced by hyperthyroidism and dysthyroidism[38] shows the danger of generalizations. The metabolic disorders and the disturbances of the vegetative nervous system described

[38] See Wertheimer and Lyman (92). The hallucinatory psychosis with fear and confusion (case 7) was operated upon and finally took a chronic more or less typical schizophrenic course.

in affective and in schizophrenic psychoses may well be more directly related to the morphological constitution as a whole than to the psychopathological reaction itself.

The problem of morphological changes in endocrine disorders has not been approached with sufficiently accurate anthropometric methods. Instead, impressionistic morphological traits were translated into a highly speculative endocrinological language. The same holds true of the endocrinological interpretation of psychological types; L. Berman's recent book "The glands regulating personality" is an example. Even in the relatively simple sphere of eunuchoid personalities, no unitary decision has been reached. Fränkel (22) finds in the eunuchoid character the most multiform qualities. Scherk (78) finds among eunuchoids a "unitary mental structure" which can be clearly defined and delimited as a whole, and shows variations only as to degree—in other words, a psychological "type." Kretschmer (46) sees close analogies between eunuchoids and schizoid psychopathic personalities. It is evidently for such reasons that Lipschütz (51) says: "The deeper one enters into the problem of connections between inner secretions and personality, the clearer it becomes that every progress in this special question is dependent on a more thorough study of the typological problem itself" (p. 253).

A fundamental connection between body architecture and mental disease, or disposition to mental disease, is inconceivable unless one would believe in a fate-like transmission of both. A genetic explanation would have to take into account constitutional signs in the physiological sphere. The possible variations of simple physiological activities in different individuals have received very little scientific attention. According to Wuth (93) the carbohydrate tolerance, endogenic purin and creatinin excretion, and nitrogen minimum show a remarkable individual constancy and may well have correlations with other constitutional signs. From the facts that have been definitely established it seems, however, more than likely that the individual variations of even the most simple physiological reactions like the knee-jerk or the respiration volume, are far greater than the variation of certain morphological signs. Rautmann (71) has obtained from soldiers and students data which show this for a number of

morphological and physiological signs. The smaller the range of individual variations for one sign, the greater significance may be attached to it, if it shows in an individual or a group of individuals values outside the usual small range of variations. Apart from their great variability, however, the correlations of physiological reactions among themselves are as yet very uncertain, even in the field of the vegetative nervous system, which seemed to offer most hopes for the establishment of physiological types. From the physiology of respiration Rutz (1911) (quoted by Gruhle (27)) has distinguished four respiration types, with supposedly characteristic mental make-up:

1. The abdominal type
2. The ascending type
3. The descending type
4. The thoracic type

but although his observations are doubtless interesting, these types are somewhat speculative and construed.

The concept of the unity of the organism has been used in recent times by biologists and psychiatrists. Yet in the field of investigation of the human constitution the limitation of methods makes desirable the distinction of the morphological, physiological or psychological aspect of constitution. No attempt to explain the human organism as a whole can be complete which leaves out any one of them. Attempts have been made very frequently to make short-cuts and to express psychological reactions in terms of morphology or phsyiology. Graves (26), one of the last scientists imbued with the spirit of Gall, has taken refuge with the shoulder-blade for the estimation of human adaptability, because he wrongly believes that "we cannot subject the mental side of man to direct observation." The uselessness and danger of translating psychological facts immediately into a physiological language "which at present is entirely arbitrary" (36) has been often pointed out, especially by Janet and Adolf Meyer. The modern psychoanalytic mechanism of compensation has been directly related by Lewis (49) to "somatic compensatory changes of growth" in paranoid schizophrenic patients. The constitutional types of Kretschmer have already led with some authors to a terminology in which morphological and psychiatric expressions are mixed. Liepmann (50)

contrasts the "pyknic" type with the "schizoid" type (p. 168). In a similar way Naccarati (63) on page 256 states that Kretschmer's "pyknic types" correspond to the macrosplanchnic, the "schizoid types" to the microsplanchnic; or, in another place (62), "Too much mental repression and the quick muscular exhaustion make the hyperthyroid a prospective candidate for the asthenic forms of psychoneurosis." This latter passage illustrates well the mixture of psychodynamic, physiological, endocrinological and psychiatric-clinical language. Pende (65) has elaborated a speculative simplified scheme in which he reduces "morpho-physiological" and psychological facts to two biotypes (p. 48).

Although we must assume that structure and function have a definite relationship also in the sphere of psychobiological integration, both the extent and the nature of this relationship are as yet unknown and a field for investigation. To use morphological constitutional signs for psychiatric diagnosis or prognosis is premature, although the incidence of dysplastic features may perhaps be used with some caution in differential diagnosis. Nevertheless, the possibility of morphological and psychopathological correlations opens a field for investigation with a bearing upon many lines, and seems to have a significance which no thoughtful clinician can overlook.

SUMMARY

1. From a historical study of the subject of characteristic body types it becomes clear that:

a. Attempts to describe body types have been made frequently; the early studies were mixtures of morphological, physiological and psychological features.

b. Medical studies of the subject were undertaken primarily with the view of correlating body types with disposition to disease as in de Giovanni's investigations.

c. In psychiatry anthropological studies were influenced largely by the doctrine of degeneracy (Morel, Lombroso).

d. A comparison of attempts to formulate morphological types shows a remarkable uniformity of the characteristic body forms aimed at by different investigators. These various classifications have not all been

collected and compared before, some authors having been overlooked in the literature on the subject, and almost forgotten (Carus, de Giovanni, Mills, etc.).

2. For the purpose of investigations like the present one, the human constitution is defined as the correlative unity of those morphological, physiological and psychobiological developments of the individual which are definitely more influenced by heredity than by environment.

3. From a critical review of the methods to be applied it becomes apparent that methods of observation and exact measurements have to be used; the problems of clinical anthropology are different from those of racial anthropology, and therefore the methods have to be adapted accordingly.

4. The descriptions of types given by Kretschmer are the most useful. Very similar types have been described before by other authors (de Giovanni, Manouvrier, etc.). Drawings of two young individuals of the same age are given here as illustrations of the main differences between the pyknic and asthenic types. (Plates I-V).

5. The methods of observation are clarified and simplified by the introduction of schematic diagrams, showing typical forms of trunk profile, head outline, hands, forehead, lips and chin. (Figs. 3–7).

6. From an inductive study using exact anthropometric measurements alone no morphological types were found.

7. A critical review of so-called constitutional indices shows that none of them can be put to clinical use; for example, the Pignet Index used by Henckel contains weight, which is variable in mental disease.

8. A new index using skeletal points alone is described:

$$\text{Index} = \frac{\text{Leg length} \times 10^3}{(\text{Transverse chest } D^*) \times (\text{sagittal chest } D) \times (\text{trunk height})} \times 100$$

* D represents diameter.

This index is based on de Giovanni's conception that the impression of the existence of morphological types is based on the relation of trunk volume to limb length. In the 65 patients of this study the observation types agree remarkably with the index values, showing that the extreme low index values correspond to the pyknic type and the extreme high values to the asthenic type, with the less characteristic

types between. In this way it is shown for the first time with exact anthropometric methods that there are no types in the strict sense, but only transitions which probably fit into a normal frequency curve, the extreme forms impressing one as types.

9. The clear asthenic, athletic and pyknic observation types of Kretschmer can be exactly differentiated by the use of the index; the athletic type stands between the asthenic and pyknic types, according to the index, but definitely nearer the asthenic.

10. The practical advantage of the index is that it can easily be put to clinical use.

11. The influence of race and occupation on these typical body forms seems negligible.

12. Among the patients with high index values the schizophrenic psychoses predominate over the manic-depressive psychoses; among the patients with low index values (pyknic and pyknoid) the manic-depressive psychoses predominate. The exact numerical relationship cannot yet be established on account of the influence of age and growth.

13. Cases in the affective group specified as showing schizophrenic reactions show morphological characteristics which are more frequent in the schizophrenic group.

14. Dysplastic features (anomalies in the distribution of hair and fat, marked disproportions of the body) predominate very definitely in the schizophrenic group, so that with some caution they may be used in differential diagnosis.

15. The age displacement of types is an important source of error (less significant for dysplastic features); with the percentages of body types in psychoses given by Kretschmer and other investigators using his methods, one is forced to consider that the average age of unselected manic-depressive patients is greater than that of schizophrenic patients. Kretschmer quotes Rohden: in 937 manic-depressive cases 66 per cent are pyknic; in our group of patients the percentage is only 45.5 per cent. If, however, the cases under thirty years of age are left out, the percentage is 66.6 per cent.

16. Two groups of prepsychotic personalities are contrasted with regard to the affective attitude toward the personal and social environment; those who seek satisfaction in emotional contact with others

(syntropic) and those who find satisfaction in being detached and isolated, with the emphasis of their emotional experiences not on other personalities but more on the reactions and ramifications of their own mental experiences both intellectual and imaginative (idiotropic). These characteristics do not constitute personality types, but denote antagonistic directive tendencies with regard to affective attitude.

Average index of syntropic group	217.2
Average index of idiotropic group	289.4

The pyknic habitus predominates in the syntropic group, the asthenic-athletic types in the idotropic group.

17. The endocrinological interpretations of morphological and psychological types are speculative (Pende, Berman, Naccarati, etc.); a genetic explanation of the relationship between morphology and psychopathology will, however, have to take into account constitutional signs in the physiological sphere. The limitation of methods makes desirable the distinction of the morphological, physiological and psychological aspects of the human constitution.

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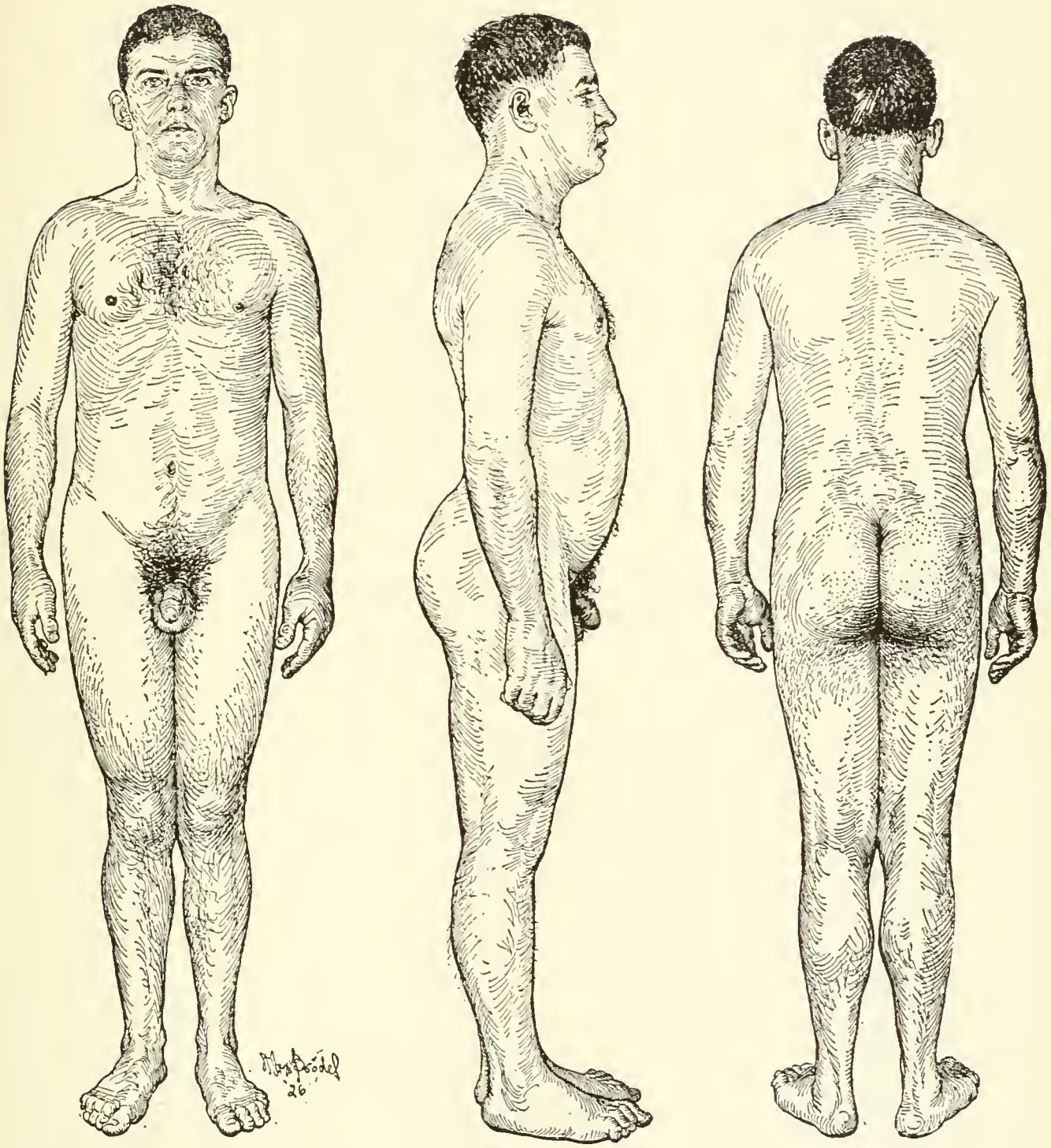
PLATE 1

Manic-depressive Psychosis, Depressive Phase. Syntropic Personality. Age Twenty-three. Pyknic Habitus. Index: 246

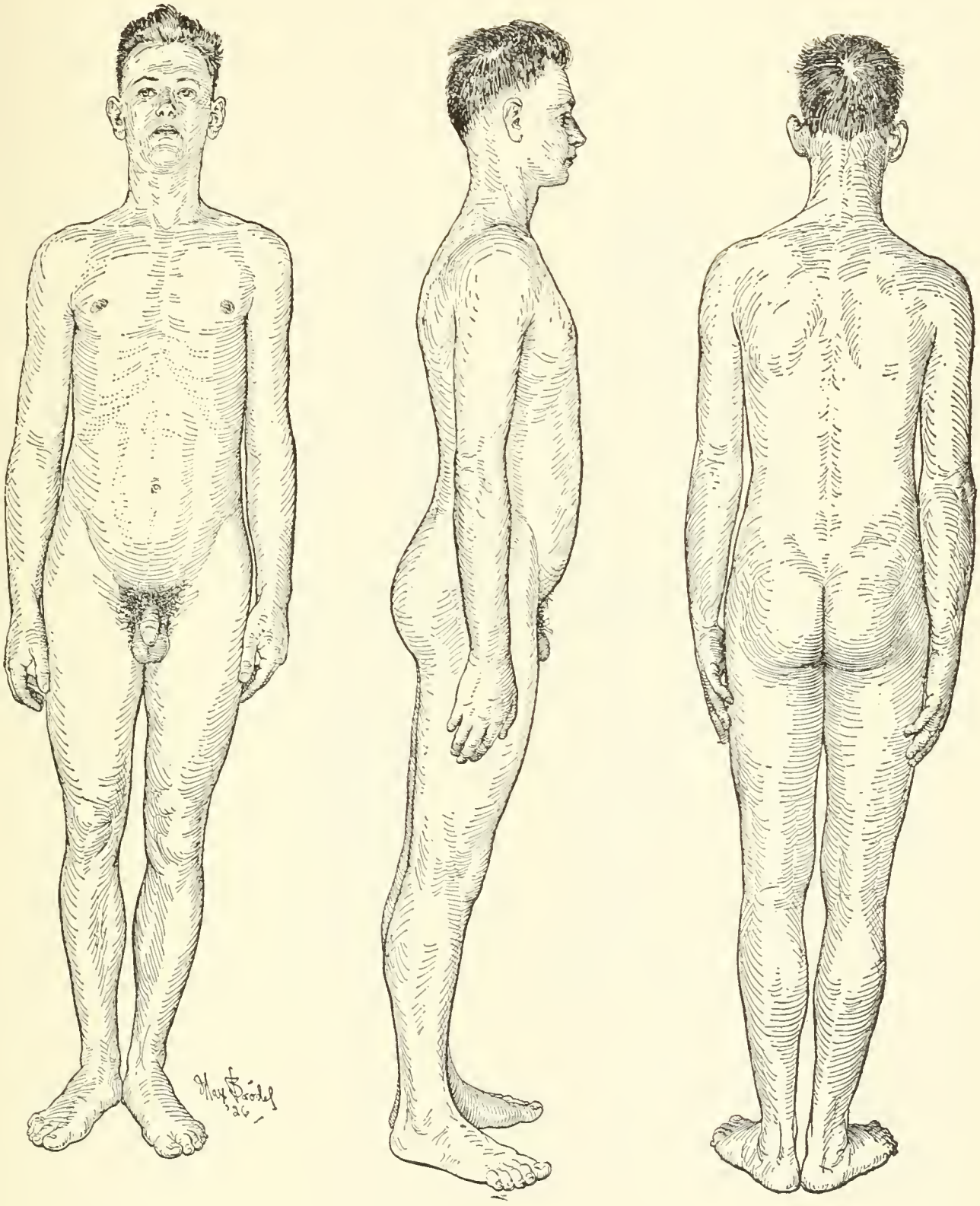


(Wertheimer and Hesketh: Physical constitution in mental disease)

PLATE 2

Hypochondriasis in Schizoid (Idiotropic) Personality. Age Twenty-three. Asthenic Habitus. Index: 305

PLATE 2



(Wertheimer and Hesketh: Physical constitution in mental disease)

PLATE 3

CONTRAST BETWEEN PYKNIC AND ASTHENIC TYPES
(Front view)

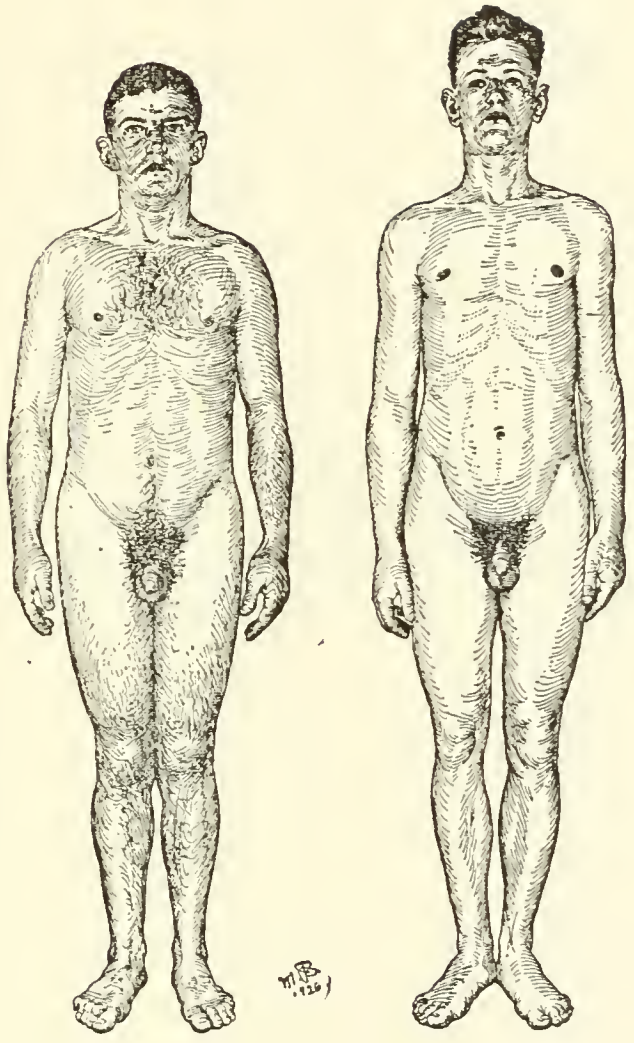


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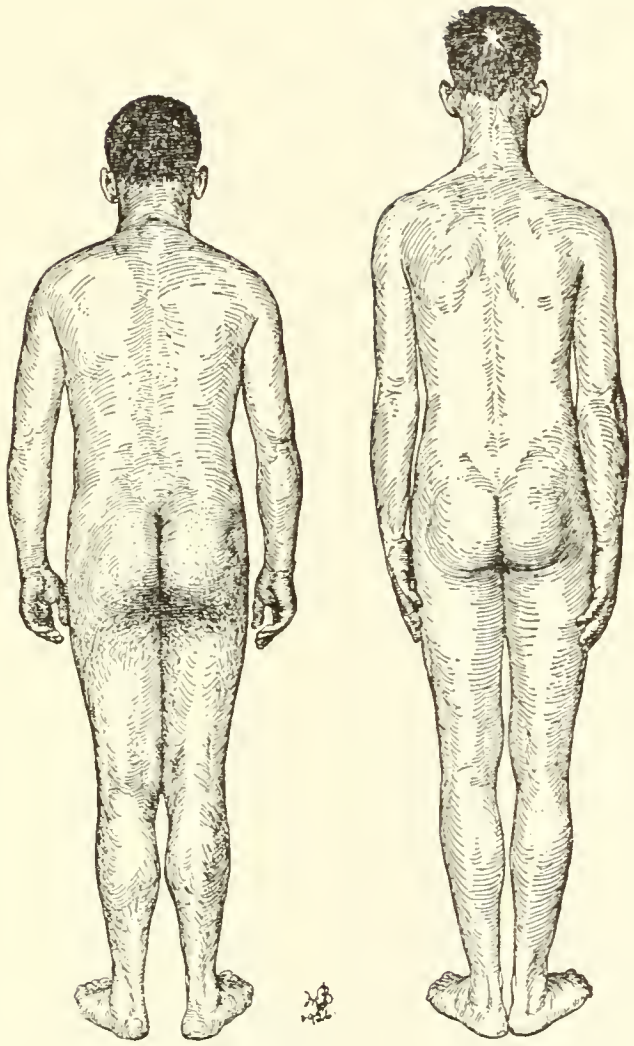
PLATE 4

CONTRAST BETWEEN PYKNIC AND ASTHENIC TYPES

(Back view)

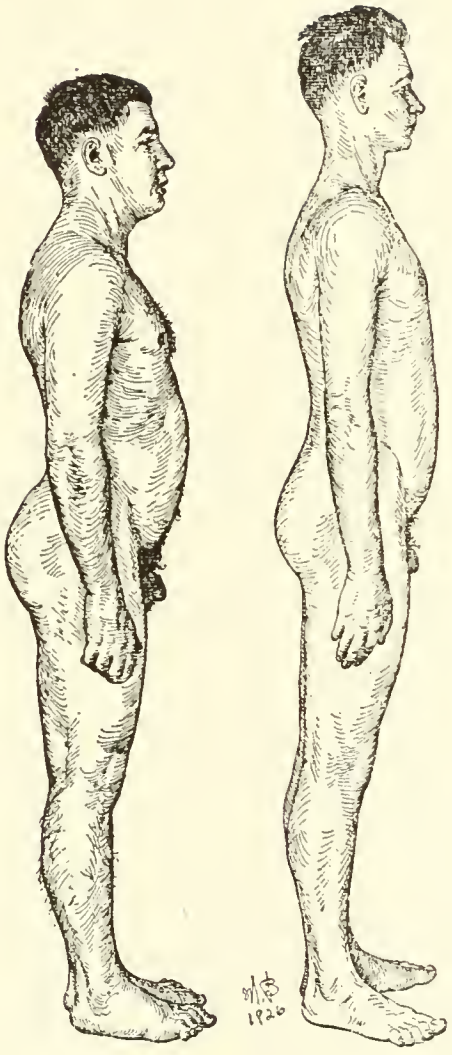


(Wertheimer and Hesketh: Physical constitution in mental disease)

PLATE 5

CONTRAST BETWEEN PYKNIC AND ASTHENIC TYPES

(Side view)



(Wertheimer and Hesketh: Physical constitution in mental disease)

IMMUNITY IN SYPHILIS

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The immunologic aspects of syphilis have engaged the attention of students of the disease ever since the earlier part of the last century when syphilis was first clearly differentiated from those other venereal infections with which it was for a time confused. Observations by the clinicians of that day soon disclosed the fact that second attacks of syphilis were extremely rare and the natural inference was drawn that one attack of the disease conferred a lasting immunity. Necessarily, however, the formulation of more precise views had to await the identification of the causal agent and the successful transmission of the infection to lower animals. These two events, which took place within such a short space of time, gave great impetus to the study of syphilis in general and were the means of shedding new light upon the question of immunity in this infection. Although considerable information has been acquired in this domain there are some phases concerning which there is not as yet complete agreement, and the field is still a fertile one for investigation.

The older experimental work has been well summarised by Neisser (1), Levaditi (2), Bruck (3) and Zinsser (4). The author has drawn freely upon their treatises and wishes to acknowledge his indebtedness to them. Indeed, were it not for the renewed interest of late in the subject and the recent acquisition of data of some importance, it is doubtful if another review of this subject would be justified.

NATURAL IMMUNITY

Species immunity

Syphilis, as is well known, occurs as a naturally acquired infection in man only. It has not been observed in nature in any other species. While there does occur an infection of the genitalia of rabbits with spiral organisms which so closely resemble the treponeme of syphilis, both in morphology and motility, as to make differentiation from the latter very difficult if not impossible, nevertheless the marked dif-

ference in the clinical picture of the two infections and the absence of any immunologic relationship between the etiological agents make it certain that they must be regarded as different disease entities. There is no reason to believe at the present time that the venereal spirochetosis of rabbits which is caused by *Treponema cuniculi* is other than a local superficial infection of the genitalia of these animals. It may then be safely affirmed that in nature syphilis is a disease affecting human beings alone.[1]

Under experimental conditions, however, the infection can be transmitted to monkeys and rabbits and can be propagated indefinitely in these species. Attempts have naturally been made to produce the disease in a number of other animal species (guinea pigs, rats, mice, cats, dogs, sheep, goats and birds) but, while successful results have been claimed in a few isolated instances, it has not been possible to carry the infection on in series and for all practical purposes these species may be said to be insusceptible.[2]

The experimental infection in both monkeys and rabbits corresponds remarkably closely with the natural infection in man, indeed perhaps more closely than most other experimental infections. Thus, with selected virus, it is possible to produce in both of these species a chronic relapsing infection characterised by (*a*) the evolution, after a suitable incubation period, of a local lesion at the portal of entry (chancre), followed by (*b*) regional lymph node involvement and (*c*) general dissemination of the organisms throughout the body by way of the circulatory system, and (*d*) attended by the development, in rabbits at least, of characteristic metastatic lesions of skin, mucous membrane, periosteum, bone and eye, although these lesions occur much less frequently in rabbits than in human beings. It has, furthermore, been demonstrated that both monkeys and rabbits exhibit the phenomenon of latency in that the virus may be demonstrated to be present in one

[1] It is of interest in this connection that some of the early explorers of South America were of the opinion that syphilis existed in the lamas of that country and that the infection was originally acquired by the natives from these animals.

[2] Recently two South American workers, Jáuregui and Lanceotti (161), reported the successful transmission of syphilis from man to lamas. These investigators satisfied themselves that they were able to reproduce all the essential features of syphilis as seen in man, even the late cardio-vascular and neurological lesions, as well as congenital syphilis. It is to be hoped that such an extraordinary achievement will be confirmed.

or more internal organs or in the lymph nodes as late as two years after inoculation, although the animal may show no clinical signs of infection. The tendency of rabbits infected with certain well-known strains of *T. pallidum* to exhibit relapsing lesions and to show a positive Wassermann reaction in their blood serum at the height of the initial manifestations of the disease serves to emphasize the essential similarity between the experimental infection and the natural disease in man, at least during the first two years of its course.

It must be admitted that as yet it has not been possible to produce, either in monkeys or in rabbits, lesions which correspond to those seen late in the course of the infection in man. Extensive syphilitic disease of the aorta or central nervous system has not been encountered in the experimental animal thus far, possibly because the individual animals have not been kept under observation for a sufficiently long period of time, although Plaut and Mulzer (5) consider that they have been able to demonstrate involvement of the central nervous system in rabbits with the production of microscopic lesions, and Brown and Pearce (6) have recently described syphilitic myocarditis in the same species. Undoubted instances of hereditary syphilis in animals have not been reported. The experiments carried out by Uhlenhuth and Mulzer (7) along this line involved experimental conditions so far removed from those of the natural infection as not to warrant any definite conclusions other than that *T. pallidum* may, when inoculated intravenously in a pregnant rabbit, pass the placental barrier and invade the circulation of the fetus. Much more significant in this respect are the recent observations of Brown and Pearce (8) who demonstrated the occurrence of a high degree of developmental defects in the offspring of syphilitic female rabbits. They were not, however, able to demonstrate the presence of the treponemes in the young.

It is possible to say, therefore, that both monkeys and rabbits are susceptible to syphilitic infection in that with virus obtained from human sources it is possible to produce an infection which in most of its essentials corresponds with syphilis as it usually manifests itself in man during the first years of the disease. The fact that the generalized lesions of the experimental disease are, as a rule, fewer in number than those usually observed in man, and differ to some extent in dis-

tribution and gross appearance, does not, to my mind, invalidate the fundamental similarity between the infection as observed in man and that produced in monkeys and rabbits. It is because of this essential similarity that deductions drawn from the experimental disease are of value in the study of immunity in syphilis.

Racial immunity

No evidence has been adduced to show that there is any race of human beings which enjoys immunity to syphilis. The most that has been claimed in this respect is that some race stocks react somewhat differently toward syphilitic infection than do others. Thus, Rotschuh (9) found severe syphilis among whites and negroes in Central America although in the Indians living in the same localities syphilis ran a surprisingly mild course, and Quennec (10) found syphilis in Africa more severe in the Europeans, Arabs and Hindus than among the negroes. Statements such as these require extensive opportunities for observation if they are to be accepted at their face value.

The tendency of negroes to exhibit syphilitic lesions of the cardiovascular system has been cited as an indication of racial predisposition to this type of reaction, but as there are so many other factors which might conceivably be regarded as entering into the development of syphilitic lesions of the cardiovascular system, opinion on this point must be guarded.

Neisser found some varieties of monkeys more susceptible to the experimental infection than others. In general, the "higher" apes, such as the chimpanzee, reacted to the virus in a manner more nearly approaching that exhibited by man, while in "lower" apes, such as the Macaques, the disease was less easily produced and, on the whole, milder.

Thus far, no particular breed of rabbits has been shown to be insusceptible to the experimental infection, nor, on the other hand, more susceptible than any other breed.

Individual immunity

While individual variations in reaction to syphilitic infection do occur in man, monkeys and rabbits, there is no evidence to show that

there exist individuals who are naturally immune to syphilis. In all probability all human beings are susceptible to the infection, but may exhibit marked differences in the manner of reacting to it. The cases of so-called malignant syphilis have been shown to be instances of increased tissue reaction to the virus and are not to be attributed to an exalted virulence of the invading parasite (Neisser).

ACQUIRED IMMUNITY

Active immunity naturally acquired

The extreme rarity of second attacks of syphilis in human beings was recorded in observations upon local outbreaks of the disease as early as the first years of the nineteenth century (11). Ricord (12) noted this infrequency and, through his insistence upon it, was one of the first to lay the foundation for the conception of a true and lasting immunity in syphilis, acquired as a result of the infection. This conception placed syphilis in the category of many other infections in which one attack confers a lasting resistance to subsequent attacks, and dominated syphilology for many years. It derived strong support from the many purposeful inoculations made upon human beings during the middle of the last century. While, as Zinsser has pointed out, much of the older information thus acquired is of limited value because of the difficulty at that time of differentiating chancre from chancroid, still it would be unfair to the clinical acumen of the syphilologists of those days to dismiss, upon the grounds of faulty diagnosis, the really large amount of evidence assembled by them. Many of the reinoculation experiments performed by these men upon their patients were, to judge from the case notes, very carefully observed, and it should be noted that the clinical recognition of syphilitic lesions had become a highly developed art at the time the experiments were made. Moreover, it must be admitted that the data derived from these numerous clinical experiments have not perhaps been contradicted by the experimental work carried out since the discovery of the *Treponema pallidum*. For this reason and because of the historical interest, it has seemed advisable to give a résumé of the work that antedated the discovery of the cause of syphilis and its transmission to lower animals. We shall attempt to present first the information

obtained prior to what may, for the sake of convenience, be termed the modern experimental era, and then, that acquired since the beginning of the latter period.

It would appear that John Hunter (13) was the first to observe that when the skin of a person with manifest generalised syphilis is scarified and inoculated with material from a secondary syphilitic lesion, no lesion is produced at the site of scarification. In view of its historical interest the account of the experiment is deemed worthy of quoting in Hunter's own words.

> A man had been affected with the venereal disease a long time and had been several times salivated, but the disease still broke out anew. He was taken into St. George's Hospital affected with a number of pocky sores; and before I put him under a mercurial course I made the following experiment: I took some matter from one of the sores upon the point of a lancet, and made three small wounds upon the back, where the skin was smooth and sound, deep enough to draw blood. I made a wound similar to the other three, with a clean lancet, the four wounds making a quadrangle; but all the wounds healed up, and none of them ever appeared after.
>
> This experiment I have repeated more than once, and with the same results; it shows that a pocky person cannot be affected locally with the matter proceeding from the sore produced by the lues venerea.

Hunter drew a further and false conclusion from this experiment, namely, that the generalised lesions of syphilis are not infectious. In spite of this erroneous deduction the observation must stand as affording the first experimental evidence of acquired resistance in syphilis.

The numerous auto-inoculation experiments upon human beings conducted in the first half of the nineteenth century showed clearly that this refractory state toward a second infection develops comparatively early in the course of the disease. Ricord (14) performed an enormous series of these experiments upon patients with genital lesions and found that material taken from a hard chancre and inoculated elsewhere into the skin of the same patient failed to produce any lesion at the site of inoculation. On the other hand, additional soft chancres could be readily produced by inoculating the skin of a patient with material taken from his own soft chancre. These observations were abundantly confirmed by other syphilologists of the time, notably Clerc (15) and Rollet. It was clearly shown that this resistance to

auto-infection which patients with hard chancres exhibited, was well developed before the appearance of generalised lesions. Indeed, so firmly was the principle established that wide-spread use was made of it in the differential diagnosis between chancre and chancroid. Thus it came about that before the year 1865 literally thousands of purposeful reinoculation experiments had been performed upon syphilitic individuals in the early stages of their infection.

Rollet has summarized all this work and his conclusions are of interest for they give us an accurate idea of the then-existing (1865) state of knowledge concerning this subject. Says Rollet,

> If reinoculation is possible before the appearance of syphilis, when it is in the stage of incubation, it is no longer so at the time when the primary lesion appears: when the chancre is manifest, when it commences to develop, it is irreinoculable, and it preserves this same character of irreinoculability during the entire period of its evolution (experiments of Nodet).
>
> From that time on immunity is an accomplished fact: one finds it in secondary syphilis to the same extent as in primary syphilis; it is also found in tertiary syphilis and even in old syphilitics several years after the disappearance of every lesion (experiments of Baerensprung). . . . . That is the general rule. As for the exceptions, however rare they may be, they show us that in some cases, in the primary as well as in the secondary stage of the disease, this immunity cannot be absolute. Reinoculations have been made with success, but in a very small number of cases, and the result, which, in order to be complete, should have reproduced the regular course of the disease, has always been but slightly pronounced.[3]

Thus matters stood in 1866 and for some time thereafter, as evidenced by the summary of the situation by Mauriac, (16) who in 1883 wrote as follows:

> 1. Reinoculation is possible before the appearance of the chancre and during the first incubation, at least up to the twenty-second day of this period.
>
> 2. It is no longer possible when the chancre begins to develop.
>
> 3. During the entire evolution of the chancre the immunity is an accomplished fact ("un fait accompli").

[3] Writer's translation.

4. This immunity persists in the secondary period to the same extent as in the primary period.

5. One finds it also in tertiary syphilis and even in old syphilitic patients many years after the disappearance of every manifest lesion.

6. In very exceptional instances reinoculations have been made with success, but without the characteristic adenopathies or the cutaneous, mucous or other lesions which always accompany the true syphilitic chancre.

7. The immunity, which *a priori* one would think ought to diminish with time, appears actually from experimentation (observation of Schnepf) to be less than during the secondary period.

8. A syphilitic individual would be more apt to be refractory to his own virus than to the virus from an individual who had attained a more advanced and perhaps more intense stage of the disease.

9. In general the pretended reinoculations are very hypothetical and in the experiments immunity has always been the rule in all stages of syphilis.

To all of these propositions, except number 8, Neisser gives his assent.

Even before Mauriac wrote the foregoing, instances were being reported in which persons with chancres had been successfully inoculated a second time and a syphilitic lesion produced. In general, however, in the case of these apparent exceptions to the rule reinoculation was performed soon after the appearance of the chancre and not during the latter part of the primary period. When reinoculation was performed later it was almost uniformly negative. Although Taylor (17) was successful in producing a second chancre by inoculating with the patient's own virus as late as the 14th day after the appearance of the original lesion and Pontoppidan (18) produced lesions by inoculating a patient 17 days after the appearance of the chancre, the number of successful reinoculations during this period of the disease was far below the number of failures. There seems to be general agreement that the lesions produced by auto-inoculation were not as characteristic as the original chancre.

These older experiments, granting the clinical diagnosis was correct, showed that the refractory state is not present in man before the appearance of the chancre but is manifest shortly after the primary lesion is established. Toward the end of the primary stage and during

the period of manifest secondary lesions the refractory stage is practically complete and universal, according to the older work.

It is difficult to form an opinion from the older literature as to the susceptibility of patients with late syphilitic lesions to a new infection, because there are so few observations or experiments on record. Occasional instances of reinfection in patients known to have had syphilis previously have been reported but the authenticity of these has been criticised. Auto-inoculations upon the bearers of tertiary lesions proved negative. Schnepf (19) in one instance apparently succeeded in reinfecting a syphilitic individual showing numerous gummata with virus from another patient. The most that can be said in this respect is that there are on record in the older literature a very small number of observations which indicate that in exceptional instances a person infected with syphilis several years previously may acquire a second infection. These cases must be regarded as constituting exceptions to, rather than examples of a general rule.

Even if one were inclined to dismiss all the evidence for the existence of acquired immunity in syphilis accumulated by the clinical investigators of the last century, on the grounds that accurate diagnosis was impossible through lack of knowledge of the causal agent of the disease, one would not be justified in dismissing the evidence obtained by purposeful inoculations performed upon syphilitic human beings in the years following the discovery of the *Treponema pallidum*, any more than one can dismiss the information obtained from study of the experimental disease in the lower animals. The era following the recognition of the causal agent of the disease and the successful reproduction of the infection in animals has indeed proved to be a most fruitful one and the facts acquired during that time have contributed enormously to our knowledge of the subject. We shall approach this phase of the work by considering what takes place in human beings, monkeys and rabbits at different periods, more or less arbitrarily chosen, in the course of the infection.

1. Incubation period. a. Man. The writer has been unable to find any account of experiments performed since 1905 upon human beings during the first incubation period before the appearance of the primary lesion, although there were a few such experiments in the preceding century.

b. Monkeys. Metschnikoff and Roux (20) succeeded in producing lesions in an orang-outang with a second inoculation performed 10 days after the first inoculation, which also resulted in the production of a lesion. The incubation period of the second lesion was shorter than than that of the first by 6 days. Finger and Landsteiner (21) in 5 experiments upon rhesus monkeys succeeded in each instance in producing second infections when the second inoculation was performed 9 to 14 days after the first. The average incubation period of the second chancre was 7 days shorter than that of the first. Similar results were obtained by Neisser in 9 apes when the second inoculation was made during the first incubation period, even if an interval as long as 51 days elapsed between the first and second inoculations. The incubation period of the second lesion was not always shorter than that of the first. Kraus and Volk (22) also succeeded in producing superinfections in apes by inoculating a second time as late as 23 days after the first inoculaton and before the first chancre had appeared. They did not always find a shorter incubation period for the second chancre but did note that such lesions were not typical and tended to be smaller and to heal more rapidly than those produced by the first inoculation.

c. Rabbits. Truffi (23) found that second infections could be produced with considerable regularity in rabbits if inoculation were performed during the first incubation period and Nichols (24) stated that "rabbits can be infected in both testicles if inoculated at the same time or within one to two weeks of each other."

Thus the experimental work on apes and rabbits supports the older clinical experiments in that it shows that during the first incubation period, up to the time of the appearance of the chancre, the host is still susceptible to a second infection introduced by scarification of the skin, and can be made to develop a second chancre. There is some indication, from the shortening of the incubation period of the second lesion in some experiments, that the host is already developing a partial resistance against foreign virus.

2. Period of early lesions. By this term is understood the period elapsing between the appearance of the chancre and the healing of the secondary lesions. As is well known this is the period during which the maximum dissemination of organisms is taking place. It also

includes the interval during which the body manifests its first defensive reaction toward the invaders, as exhibited in the spontaneous healing of lesions. While the actual time limits of the period are perhaps better appreciated in man, they are of course not sharply defined but for purposes of discussion this period of the infection may be singled out from the others. It is far less easy to delimit it in the lower animals, however, because of the relative scarcity of secondary lesions. For that reason when we come to discuss what takes place in monkeys and rabbits we shall have to rely upon time intervals expressed in days after inoculation and chosen somewhat arbitrarily.

a. Man. That the syphilitic individual during this period of his infection gradually acquires a resistance to his own or foreign virus inoculated upon the scarified skin has been clearly demonstrated by the experiments of Queyrat, and by Levaditi and his co-workers.

Queyrat (25) conducted a series of 19 auto-inoculations by scarification of the skin of patients presenting chancres and obtained 10 positive results. Control scarifications with sterile instruments were performed in each instance. No lesions were produced if the original chancre was older than 15 days. The average incubation period of these auto-inoculation products was about 13 days. The lesions which he produced contained numerous spirochetes but he himself recognized that they did not present the typical appearance of chancres and he spoke of them as "abortive chancres." He regarded them as having been modified by the pre-existing syphilitic infection. Auto-inoculations by Nobl (25a) in 8 patients with syphilis prior to the outbreak of secondary lesions all proved negative.

Levaditi, La Roche and Yamanouchi (26) compared the results of auto-inoculation with the occurrence of the Wassermann reaction in 6 experiments upon human beings with syphilitic chancres. They succeeded in producing a second chancre in three patients with primary lesions 4 to 8 days old and in all of these the reaction was negative. In the remaining 3 patients with chancres 8–9 days old reinoculations were unsuccessful and in two of these the Wassermann reaction was positive. They attach considerable importance to this coexistence between the development of the resistant state and the development of a positive Wassermann reaction in the blood serum.

These experiments of Queyrat, of Levaditi and his co-workers and

of Nobl would indicate that a high degree of resistance of the skin toward a second syphilitic infection develops in man soon after the appearance of the primary lesion. The observations of Finger and Landsteiner and of Ehrmann upon patients with secondary syphilis would however indicate that this resistance is not absolute.

Finger and Landsteiner (27) found that if they introduced relatively large amounts of syphilitic virus into pockets made in the deeper layers of the skin of patients with manifest secondary cutaneous lesions, they were able to produce lesions which did not present the clinical characteristics of a chancre but closely simulated the lesions which the patient himself was exhibiting at the time. Control inoculations with non-syphilitic material or with heat-killed syphilitic virus were negative. In their hands these inoculations were more successful when performed before the appearance of secondary lesions than when carried out later. Thus in 27 patients inoculated in the second incubation period lesions developed in 24, or almost 90 per cent, whereas in 40 patients inoculated after the appearance of secondary lesions the number showing lesions amounted to 27, or 66 per cent.

Ehrmann (28) inoculated a series of 45 syphilitics who presented "ulcero-crustaceous papules," with material containing active syphilitic virus and obtained in each instance what he regarded as a syphilitic lesion. The lesions produced were certainly not chancres; they were described as scaly or crusted papules and were not followed by regional lymphadenitis. This series of experiments seems to have been carefully controlled to exclude the possibility of trauma alone being responsible for the production of the lesion; nevertheless Neisser criticised them and expressed considerable doubt as to whether true syphilomata were produced. The conditions under which the inoculations were carried out were such as not to exclude the possibility of secondary infection playing a rôle in the pathogenesis of the lesions. Somewhat similar but less uniform results have recently been reported by Cappelli (29).

It seems clear that characteristic primary lesions were not produced in the auto-inoculation experiments of either Ehrmann or Finger and Landsteiner. About all that one would be justified in concluding from them would be that the skin of patients with secondary syphilis is not uniformly and completely indifferent to new syphilitic virus in-

troduced under certain special conditions. Zinsser is undoubtedly correct in his contention that the observations do not invalidate the conclusion that "there is a resistance at this stage higher than that of the normal subject."

b. Monkeys. The experimental work on monkeys has yielded results that are in strict accord with those obtained in the human experiments. Metschnikoff and Roux (30) succeeded in superinfecting apes as late as 10 days after the appearance of the chancre. Finger and Landsteiner in 9 experiments with rhesus monkeys succeeded in producing second infections in 6 instances when the second inoculation was made not later than 5 days after the appearance of the first chancre. They noted a shortening of the incubation period of the second chancre and a diminution in the size of the lesion which they attributed to the influence exerted by the first infection. Kraus and Volk succeeded in producing second chancres in syphilitic apes with primary lesions 2, 4, 6 and 11 days old but failed in 6 instances in which inoculations were made 2, 4, 5, 7, 10 and 19 days after the appearance of the first chancre. They failed to note a uniform shortening of the incubation period in the case of the second infection.

Neisser successfully reinoculated 2 apes on the day the original primary lesion appeared. He obtained successful reinoculations in 9 apes as against unsuccessful reinoculations in 135, when the second infection was performed after the appearance of the first chancre. The percentage of successful reinoculations was greatest when the attempt was made before the eightieth day of the disease, and was almost nil when the attempt was made at a later period.

c. Rabbits. In the foregoing experiments upon human beings and monkeys the inoculations were almost always made by the cutaneous or subcutaneous route. When we come to consider the state of affairs in rabbits we have to take into account other modes of inoculation. The first authentic transmission of syphilis to the rabbit was accomplished by inoculation into the anterior chamber of the eye, with the production of keratitis. In the earlier reinoculation experiments this method of inoculation was employed and it was not until later that the testicular and subscrotal methods were adopted. The subcutaneous and intracutaneous methods have not been employed in rabbits to anything like the extent that they have been used in apes. From the

work of several investigators it is clear that in rabbits the results obtained from reinoculation vary to some extent, depending upon the site of the original and also of the second inoculation. This point, as Tomasczewski has emphasized, should be kept clearly in mind when evaluating any reinfection experiments in syphilis, and for that reason we shall discuss first the information obtained by inoculation of the anterior chamber of the eye, and then that obtained by the more common intratesticular and subscrotal methods of inoculation, in untreated animals only.

A. First and second inoculations in the anterior chamber of the eye. Bertarelli was the first to carry out reinoculation experiments in syphilitic rabbits. Both first and second inoculations were made into the anterior chamber of the eye. In his first experiments (31) he used virus from human sources, hence heterologous, and in three rabbits which had previously had unilateral syphilitic keratitis he was unable to produce disease of the opposite (unaffected) cornea upon subsequent inoculation. In a second series of experiments (32) in which syphilitic virus was used that had been passed through rabbits and hence was presumably homologous, he inoculated corneas in which syphilitic keratitis had previously been present and in 1 of 4 animals keratitis developed. From these experiments he concluded that there is no absolute local immunity for corneal syphilis of rabbits. Truffi (33) inoculated one anterior chamber and both scrota of a rabbit and 147 days later, after the lesions had healed, reinoculated both anterior chambers and both scrota and obtained a negative result.

On the other hand, Purckhauer (34) obtained 3 successful reinoculations in rabbits that had had a syphilitic keratitis previously. He does not state whether or not he obtained any negative results and he concludes that corneal syphilis does not produce any immunity towards a second infection. From this conclusion he deduces that no generalisation of the syphilitic virus takes place in rabbits after ocular inoculation. Colombo (35) stated that a rabbit's eye, in which syphilitic keratitis has been produced and which has apparently healed spontaneously, can be reinoculated with success. Uhlenhuth and Weidanz (36) found that syphilitic keratitis of one eye does not, as a rule, protect the other cornea against infection, whether or not the diseased eye is enucleated prior to inoculation.

Unfortunately many of the details of the earlier experiments have been omitted from the authors' papers so that it is a well nigh hopeless task to attempt to analyse the conflicting results obtained by the various workers. About all that one can conclude with safety from this material is that some rabbits with healed corneal syphilis are resistant to a second ocular inoculation, while others are not; in other words, that the refractory state is not universal so far as the cornea is concerned.

B. First inoculation in the anterior chamber, second inoculation testicular or subscrotal. Uhlenhuth and Mulzer (37) inoculated the anterior chambers of the eyes of 4 rabbits with syphilitic virus and in all a bilateral keratitis developed. After the keratitis had healed spontaneously the animals were inoculated intratesticularly with virus from syphilitic rabbits and in 3 of the 4 orchitis subsequently developed. In a similar series of experiments Tomasczewski (38) obtained 5 successful reinoculations in 6 animals with healed syphilitic keratitis, the second inoculation being made with rabbit virus in the subscrotal tissue from 2 to 128 days after the keratitis had healed. In 17 rabbits with pre-existing syphilitic keratitis Adachi (39) obtained 8 successful reinoculations using the subscrotal method 41 to 120 days after the first infection.

From these experiments it would appear that syphilitic keratitis in the rabbit does not confer protection against a second infection introduced into the testis or scrotum.

C. First inoculation testicular or subscrotal, second inoculation in the anterior chamber. The results obtained when the first inoculation of syphilitic virus is made into the testis or the scrotum of the rabbit and the second into the anterior chamber of the eye have been conflicting. Uhlenhuth and Mulzer inoculated 11 rabbits intratesticularly, some unilaterally and some bilaterally, and subsequently inoculated them a second time by the intraocular route. The interval between first and second inoculations varied from 59 to 170 days: in some of the animals the orchitis was still active at the time the second inoculation was performed, in others it had healed. In only 1 of the 11 animals did the second inoculation result in a keratitis, in this instance after an incubation period of 25 days. Tomasczewski, on the other hand, obtained almost the opposite result. He inocu-

lated 21 rabbits subscrotally and in all of them syphilitic lesions developed. Second infections were then performed by the intraocular route from 39 to 105 days after the first inoculation. In 16 of the 21 animals, or 76 per cent, keratitis developed from 4 to 6 weeks after inoculation. He states that this incidence of positive reinoculations is almost identical with the incidence of infection obtained by him in normal rabbits inoculated in the same manner. More recently Frei (40) inoculated 25 rabbits subscrotally or intratesticularly, or by both methods and, after the lesions had healed, reinoculated by the intraocular route. The interval between first and second inoculations varied from 2 months to $2\frac{1}{4}$ years. He obtained only 4 successful reinoculation results, or 16 per cent. In his experiments he employed homologous virus for the second inoculation. In a similar experiment Adachi (39) obtained 5 successful reinoculations in 18 rabbits.

It is difficult, if not impossible, to explain these conflicting results. The high percentage of negative results obtained by Frei, as contrasted with the numerous positive results of Tomasczewski, might be explained on the basis of longer interval between first and second inoculations but in that case the negative results obtained by Uhlenhuth and Mulzer are still to be explained. Careful scrutiny of the protocols of the experiments of the latter fails to disclose any way in which their results may be brought into harmony with those of Tomasczewski.

D. First and second inoculations testicular or scrotal. In most of the studies dealing with reinfection in experimental syphilis of the rabbit, the testicular or subscrotal methods of inoculation have been employed. Truffi (41) was apparently among the first to conduct such experiments. In 1908 he inoculated a rabbit subscrotally with human virus and obtained a syphiloma which healed spontaneously. Five successive attempts at reinoculation of this animal by the same method yielded negative results. A sixth attempt, performed 514 days after the original inoculation, yielded a positive result. Altogether he reinoculated 6 rabbits and succeeded in producing a second infection in 4 instances, including the one mentioned. In these animals the second (successful) inoculation was made 12, 26, 76 and 514 days after the first. It is not clear from his article that all the negative results are recorded.

Ossola (42) reinoculated 4 syphilitic rabbits but failed to obtain a single positive result. In the case of 2 of the animals the interval between first and second inoculation was 56 and 106 days, respectively. In the case of the other 2 the time interval is not stated. Homologous virus was used for reinoculation in all 4 animals.

Uhlenhuth and Mulzer reinoculated 8 rabbits at intervals of from 53 to 178 days after the first inoculation and obtained what they regarded as successful reinoculations in 7. In 4 of these the lesion developed in the testis which had not previously been inoculated. No significant differences in the incubation period of first and second infections were noted. Reinoculations were apparently made with the same strain of treponemes. In the single instance in which a negative result was obtained the second inoculation was performed 178 days after the first.

Tomasczewski reinoculated 24 syphilitic rabbits at intervals of from 7 to 92 days with homologous virus, both first and second inoculations being made subscrotally, and obtained lesions in 18 animals. All those reinoculated before the sixty-seventh day of the disease gave positive results and none of those in which the second inoculation was performed after this time showed any signs of a second infection. The character of the lesions produced as a result of reinoculation varied; half of them were chancres and the other half papules. Tomaszcewski interprets the occurrence of papules as an evidence of changed reaction capacity on the part of the scrotal skin.

Nichols reinoculated 5 syphilitic rabbits, the second inoculum being introduced into the opposite testis from that originally inoculated, and obtained what he regarded as second infections in 3 of the animals. The originally inoculated testis had been removed prior to reinoculation in 2 of these animals. In each instance the incubation period of the second infection was longer than that of the first.

Zinsser, Hopkins and McBurney (43) reinoculated 17 syphilitic rabbits, the homologous strain being used in 4 instances and a heterologous strain in 13. Altogether, lesions containing treponemes were produced in 3 instances and in a fourth chancres were produced but treponemes were not demonstrated. In 5 of the remaining 13 "small nodules" appeared following the second infection but the authors are in doubt as to the nature of these lesions since no treponemes were

demonstrated in them. The remaining 8 animals showed nothing at all following the second inoculation. The interval between the first and second inoculation was comparatively long, ranging from 38 to 458 days. In all but one instance this interval exceeded 100 days. The 3 undoubtedly successful reinfections obtained by these observers occurred in animals reinoculated with heterologous strains.

Fournier and Schwartz (44) concluded that rabbits in which syphilitic lesions have been allowed to heal spontaneously are refractory to a second infection with the same strain.

Brown and Pearce (45) showed that if untreated rabbits with bilateral syphilitic orchitis were inoculated by intracutaneous injection with the homologous strain of treponemes 24 days after the first inoculation, slight lesions were produced at the site of the second inoculation in a few instances. The lesions produced, however, were not comparable with those seen in the control animals, but they were regarded as syphilitic by these authors.

Kolle (46), on the basis of several hundred reinoculation experiments, has stated that if a second inoculation is carried out with homologous strains of treponemes (Truffi strain) within 60 days after the first inoculation, a typical chancre may be produced in 50 to 60 per cent of the cases. If the reinoculation is performed between the sixtieth and ninetieth day, occasionally a chancre is produced, whereas if it is carried out after the ninetieth day no lesion develops. Most of his reinoculations were by the intratesticular route but some were made upon the skin of the back. Frei obtained similar results. In 8 reinoculations performed before the twenty-eighth day of the disease with homologous strains, lesions were produced in every instance: in 7 performed 49 days after inoculation the result was negative in 4 instances and positive in the remaining 3, whereas in 52 rabbits in which the second inoculation was carried out from 63 days to 4 years after the first, all of the animals were refractory to a second infection.

Reiter (47) reinoculated 19 rabbits at intervals of from 133 to 602 days after the first inoculation and obtained 3 undoubted and 1 questionable reinfection. Ten of the reinoculations were made with homologous strains and 9 with heterologous strains. All of the successful reinoculations and the questionable one were obtained with heterologous virus. Chesney and Kemp (48, 49) reinoculated 8

rabbits with the homologous strain of treponemes from 270 to 336 days after the first inoculation and in no instance were they successful in obtaining any macroscopic lesions. Adachi (50) reinoculated 31 syphilitic rabbits 151 to 531 days after the infection and obtained 7 successful reinfections, all with heterologous strains, whereas all the reinoculations with homologous strains were negative. The same investigator produced chancres in a series of rabbits by uni-lateral scrotal inoculation. At varying intervals (3 to 43 days) after the appearance of the chancre the lesion was excised and used for inoculating the opposite testis. When the excision of the original chancre was performed earlier than 18 days after its appearance (thirty-first day of the disease) the other testis could be infected, whereas if excision were carried out 23 days or more after its appearance (forty-seventh day of the disease) the result was always negative.

From the foregoing it would appear that in rabbits a first inoculation made in the testis or scrotum confers upon the same or opposite testis or scrotum, protection against a second infection, provided a sufficiently long period of time is allowed to elapse between the first and second inoculations and provided homologous strains are used throughout. Under such conditions, as Kolle has pointed out, the percentage of successful second infections diminishes and almost reaches zero if the time interval between successive inoculations is increased.

The time at which this refractory state is fully established varies according to different observers, possibly due to variations in strains, but the main fact seems now to be well established. I have gathered together from the available literature all the reinoculation experiments in *untreated* rabbits with homologous strains of *T. pallidum*, where the intratesticular or subscrotal method has been employed for both first and second inoculations, and where the time interval between inoculations is expressly stated. These are summarised in table 1.

As will be seen from table 1, when the second inoculation is carried out between 20 and 60 days after the first, the percentage of successful reinoculations is 78.7; when the second inoculation is performed between the sixtieth and the ninetieth day of the disease the percentage falls to 11.4 and when it is carried out more than 90 days after the first the percentage is only 5. These figures bear out Kolle's results in untreated rabbits with remarkable accuracy.

The situation is somewhat different if heterologous strains are used for reinoculation. In that event a much higher percentage of reinfections is obtained, even if the second inoculation is performed late in the course of the disease, at a time when the rabbits would presumably be refractory to a second inoculation with homologous virus. This point has recently been emphasized by Kolle (51) although it was indicated previously by the work of Zinsser and his collaborators, of Brown and Pearce (52), and of Reiter. The results of the investigations of all

TABLE 1

Reinoculations of untreated syphilitic rabbits with homologous strains

AUTHOR	DAYS AFTER INOCULATION											
	20–60				61–90				91+			
	Number		Per cent		Number		Per cent		Number		Per cent	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Ossola	1	1	0	100					0	1	0	100
Uhlenhuth and Mulzer	2	0	100	0	2	0	100	0	3	1	75	25
Tomasczewski	11	0	100	0	1	6	14	86				
Nichols	2	2	50	50	1	0	100					
Zinsser et al									0	4	0	100
Frei	11	4	73	27	0	25	0	100	0	34	0	100
Reiter									0	10	0	100
Chesney and Kemp									0	8	0	100
Kolle			50–60				*				0	
Total	26	7	78.7	21.3	4	31	11.4	88.6	3	58	5	95

* Kolle states that in reinoculations between the sixtieth and the ninetieth day typical chancres may be produced occasionally.

these workers, where the details are available, are summarized in table 2.

Study of table 2 shows that in more than a third of the untreated rabbits reinoculated with heterologous strains late in the course of the disease, a second infection may take place. Recent work by Adachi (50) and some as yet unpublished experiments of Chesney and Kemp (53) are in accord with this finding.

The experimental observations upon man, monkeys and rabbits, therefore, show that it is possible to produce second infections by inocu-

lating the host even after the appearance of the initial lesion, provided not too long a period of time is permitted to elapse before the second inoculation is carried out. As time goes on, however, the chances of producing a second infection diminish. The precise time limits during which it is possible to produce such a second infection vary somewhat with the different animal species and probably with the individual strains. The recent work with rabbits would indicate that in syphilis the refractory state toward a second infection which does develop with time is more marked toward the homologous strain of treponemes than toward a heterologous strain. It is possible that the refractory state appears at an earlier date in man than in monkeys or

TABLE 2

Reinoculations of untreated syphilitic rabbits with heterologous strains

AUTHOR	REINOCULATIONS CARRIED OUT 90 DAYS OR MORE AFTER FIRST INOCULATION			
	Number		Per cent	
	Positive	Negative	Positive	Negative
Zinsser et al.	3*	9	25	75
Reiter	4	5	44	56
Kolle	23	28	45	55
Chesney and Kemp	7	4	63.6	36.4
Total	37	46	44.5	55.5

* One of these clinically positive but dark field negative.

rabbits but this difference may be only apparent and may possibly be explained upon the basis of differences in method of inoculation, amount of virus in the inoculum, or again upon a less rapid generalization of the virus in the lower animals. Not much weight can be given to this last-mentioned possibility, however, since Pearce and Brown (54) have demonstrated that in rabbits migration of the treponemes from the site of inoculation takes place within a very short time (48 hours) after testicular or scrotal inoculation.

3. Late period. a. Man. Purposeful inoculation experiments upon syphilitic individuals who have had their infection for years, whether or not they showed tertiary lesions, have been comparatively few in number since the discovery of *Treponema pallidum.*

Finger and Landsteiner inoculated a series of 15 patients exhibiting tertiary lesions with material from human sources rich in treponemes. Inoculations were made by introducing the virus into subepidermal pouches. Lesions developed in 13 of the subjects at the site of inoculation. These did not resemble chancres but presented the clinical appearance of tertiary lesions (cutaneous tubercles, gummata and ulcerative syphilides), and were extremely poor in spirochetes. Inoculations with heat-killed virus were negative. Salmon (55) repeated these experiments on three subjects whose syphilis was of long duration (20 to 32 years), and who were actually exhibiting tertiary lesions at the time of inoculation. Altogether 8 inoculations were made upon the 3 patients. In one of the patients two small papules developed, the others remained free of any lesions. Nothing resembling a tertiary lesion was produced. Queyrat and Pinard (56), on the other hand, were apparently successful in one instance in producing a typical ulcerative syphilide by inoculating an individual having a tertiary lesion, with a small piece of a chancre placed in an intradermal pouch. The original infection occurred 7 years previously in this case and a period of 17 days elapsed between inoculation and the development of the lesion. No treponemes could be demonstrated in the lesion at any time. Likewise Vignolo-Lutati (57) succeeded in producing a gummatous lesion by inoculating a patient showing tertiary phenomena with a portion of a primary lesion, introducing the inoculum after the manner of Finger and Landsteiner. von Poor (58) inoculated 12 syphilitic individuals exhibiting outspoken tertiary phenomena with material taken from their own lesions but in no instance did anything develop at the site of inoculation. On the other hand Cappelli (29) was successful in producing an ulcerative lesion resembling a gumma in a patient whose syphilis was of 18 years duration. Krafft-Ebing (59) inoculated 9 paretics with material from early lesions but in no instance was he able to obtain any lesions at the site of inoculation. Siemens (60) inoculated 16 paretics and 3 tabetics a number of times (65 inoculations altogether) with syphilitic material, always with negative results, and Steiner (61) and also Sagel (62) were equally unsuccessful in a number of similar experiments. One successful inoculation was, however, reported by Jahnel (63) according to Steiner. According to the account given by the latter, the patient was a paretic whose blood

and spinal fluid had become negative after unusually intensive salvarsan treatment; inoculation of the skin of the arm with syphilitic material was followed by the appearance of two small lesions in which spirochetes could be demonstrated. It is clear that this one positive result must be regarded as an exception to the rule.

So far as the foregoing experiments go, they suggest that the late syphilitic is refractory to a second infection in the sense that new syphilitic virus introduced from without is incapable of producing a chancre followed by secondary manifestations. The most that they show is an altered capacity on the part of the tissues to react to the virus. We shall return to this interesting subject later.

b. Monkeys. Metschnikoff and Roux were unable to obtain successful reinoculations in apes in the later stages of the disease, and Finger and Landsteiner were likewise unsuccessful. In the hands of the latter the animals were still refractory 10 months after the first inoculation. As stated above, the incidence of positive reinoculations obtained by Neisser in monkeys was extremely low, actually only 3 in 114 experiments, or 2.6 per cent, when the second inoculation was performed after the 80th day of the disease. He obtained negative results when the interval between first and second inoculations was as long as 650 days. His results show clearly that in apes, at least, the resistant state, once acquired, persists for almost two years and for all we know may persist even longer.

c. Rabbits. As has been shown in a preceding section, where the question was discussed in greater detail, the state of resistance toward a second infection with the homologous strain of treponemes, which rabbits acquire as a result of their first infection, is well established in nearly all instances by the 90th day of the disease and has been shown to persist for at least 4 years. (Frei.) There are exceptions to this generalization, of course, but the principle seems to hold true in the main. This resistant state is definitely more effective against homologous strains of treponemes than against heterologous strains, although to be sure it is effective against even these latter in at least half of the cases.

In general it may be stated that the reinoculation experiments upon man, monkeys and rabbits carried out in the later stages of the disease tend to show that the untreated syphilitic individual ultimately

acquires a not inconsiderable resistance to a second infection. While in certain instances lesions have been produced by inoculating patients having tertiary syphilis with syphilitic virus, nevertheless these phenomena have the characteristics of late lesions rather than chancres, and are not followed by the familiar secondary manifestations.

Summary

It may perhaps be well at this point briefly to summarize the foregoing data before passing on to more theoretical considerations as to the nature of immunity in syphilis.

The reinoculation experiments that have been carried out upon syphilitic human beings, monkeys and rabbits since the discovery of the *Treponema pallidum* all tend to show that the host gradually acquires a resistance, more or less complete, toward a second infection with the same microörganism if the disease is allowed to develop untreated. The time at which this resistant state is first manifest varies to some extent with the particular animal species under investigation but in general it may be said that it is already present to a high degree before the primary lesion has healed. It is certainly present during the period of secondary lesions and during the later years of the infection. There is no reason to believe that this acquired resistance may not persist for the balance of the life of the host in many instances, although it seems likely that it may be subject to fluctuations from time to time, and is not absolute.

There is some evidence to show that not all tissues of the body share equally in this resistance and there is good ground for the view that, in rabbits at any rate, it is more effective against homologous strains of treponemes than against heterologous strains. Above all, it is clear that the time factor is essential in the acquisition of this resistant state.

From what has just been said it must be admitted that the data derived from the modern experimental work in syphilis have not overthrown the older conception of the development of an acquired resistance, during the course of the disease, to a new infection. But, as we shall see presently, some of this later work has been instrumental in throwing light upon such interesting questions as the nature of this resistance, its distribution, and particularly its relation to persistence

of infection. This last question is of paramount importance from the standpoint of our theoretical conceptions of immunity in syphilis and in addition has an important practical bearing upon treatment and cure, as we shall see later.

CO-EXISTENCE OF INFECTION AND IMMUNITY

As a result of his studies in syphilis in apes Neisser brought forward a new conception of immunity in this infection, a conception which has dominated this field up to the present time, although recently evidence has been forthcoming which perhaps casts some doubt upon the validity of Neisser's generalizations. Briefly stated, this investigator came to the conclusion that the resistance which a syphilitic animal acquires during the course of its disease is dependent upon the persistence of active foci of syphilitic infection somewhere in its body, and disappears if these foci are eliminated. If an animal previously infected with syphilis proved refractory to a second infection that fact in Neisser's opinion indicated that the animal still harbored the first infection, whereas if it were found to be susceptible to a second infection that finding meant that the first infection had been eliminated. Accordingly Neisser took the ground that the reinoculability or non-reinoculability of an animal could be taken as evidence of the success or failure of treatment, that is to say cure or no cure. To put it a little differently, if a treated animal could be successfully reinfected it should, according to Neisser, be regarded as having been cured; if it was still refractory to a second infection it had not been cured. Neisser was firmly convinced that there is no such thing as true lasting immunity in syphilis, that is to say, an acquired immunity which persists in the absence of infection. This conception is obviously of such fundamental importance from the standpoint of both theory and practice that it becomes necessary to subject the data upon which it is based to a critical analysis. It must be clear to anyone that the matter is far from being one of academic interest only.

Neisser's conception derives its support from two sets of observations made by him; first, the determination that untreated syphilitic apes which are refractory to a second inoculation can be shown to harbor the virus in their internal organs; second, that apes treated with various antisyphilitic drugs and thought to have been cured, are at

once susceptible to a second inoculation, whereas other apes treated but thought not to have been cured remain refractory. These treated and reinoculated apes were regarded as having been cured or not, on the basis of the results of inoculation of normal animals with emulsions of the internal organs of *other* apes treated in a similar fashion. It is thus a question of comparison of results of reinoculation in one series of treated animals with the results of organ transfer in another series. It should be noted that in no instance did Neisser compare both methods in the same animal.

As regards the first point mentioned above, namely, the demonstration that untreated apes which are refractory to a second inoculation are still syphilitic, the evidence brought by Neisser is quite convincing. He removed internal organs from 29 syphilitic apes that had previously been shown to be resistant to a second inoculation and inoculated normal animals with emulsions of these organs. By this means he succeeded in demonstrating that the internal organs of 22 of these animals contained living syphilitic virus. In the case of the remaining 7 whose organs gave negative results it should be stated that many of the normal animals inoculated with this material died prematurely. It is clear from these experiments, therefore, that untreated apes that have acquired a resistance to a second syphilitic infection can be shown to harbor the virus in their internal organs in a large proportion of the cases (76 per cent in Neisser's experiments).

The second point that he makes, namely, that syphilitic apes treated and thought to have been cured are, in a high percentage of the cases, susceptible to a second inoculation with syphilitic virus, while apes treated but not cured are as a rule refractory, rests upon the results of a series of reinoculations upon 198 treated monkeys. These animals were treated with a variety of drugs and were then inoculated a second time. Second infections were produced in 107 instances (54 per cent), doubtful results were obtained in 9 (4.5 per cent), and negative results in 82 (41.5 per cent). The incidence of successful reinoculations in these *treated* animals, 54 per cent, is in striking contrast with that, approximately 6 per cent, obtained in a series of 145 *untreated* animals. Neisser assumed that those treated animals which proved to be susceptible to a second inoculation had been freed of their infection by treatment since organ transfer from other apes similarly treated

gave a large percentage of negative results. Likewise, treated animals which were thought not to have been cured, gave a high percentage of negative results on reinoculation. Again, these animals were assumed not to have been cured because of the large percentage of positive results obtained when emulsions of organs from other similarly treated animals were inoculated into normal animals. Actually, taking all his published protcols into consideration, it is found that Neisser reinoculated 198 treated apes and carried out organ transfers in 109 other treated animals. In general his protocols disclose a fairly close parallelism between positive reinoculation and negative organ transfer on the one hand, and negative reinoculation and positive organ transfer on the other. Taking all his therapeutic experiments into consideration, he obtained 54 per cent positive reinoculations and 71 per cent negative organ transfers. The animals in both of these groups he regarded as having been cured.

The crux of this argument, of course, lies in the justification for the assumption that the treated animals which later were shown to be susceptible to a second inoculation had been cured of their first infection by treatment and that those which later proved refractory to a second inoculation were not cured. Before granting that the results of organ transfer in one series of treated animals may be taken as indicative of what treatment had accomplished in another series of animals and then going on to draw deductions therefrom, one would want to know how many strictly comparable experiments there were. In other words, how many animals treated with *equivalent amounts of the same drug at the same time* were subjected to the reinoculation test on the one hand and how many to the organ transfer test on the other? Obviously if different dosages were used in the two groups where different criteria were employed, one would not be in a position to compare the results of treatment in these groups and hence one could not say with absolute certainty whether or not infection had been eliminated.

When the protocols of Neisser's experiments are examined from this standpoint one is disappointed to find that in many instances the reinoculation experiments and organ transfer experiments are not strictly comparable since different dosages were employed. Indeed, although he carried out the reinoculation test upon 198 animals treated after infection had been established, and the organ transfer test upon

87 other treated animals I have been able to find only 13 instances comprising observations upon a total of 90 animals, where the two tests were conducted upon series of animals treated with equivalent doses of the same drug. Moreover, it is not possible to determine from these otherwise strictly comparable experiments, whether the treatment was begun at corresponding periods in the course of the

TABLE 3

Comparison of reinoculations and "organprüfung" in treated apes (Neisser)

TABLE	PAGE	REINOCULATION			"ORGANPRÜFUNG"		
		Negative (no cure)	Doubtful	Positive (cure)	Negative (cure)	Doubtful	Positive (no cure)
XXXIV	266	1		5	3		
			1	4	1		
		1	1	2	5	1	
		1			1		
				4	1		
				1	1		
				2	1		
XLI	273			4	1		
				3	2		
XLIV	275	1		2	1		
LVI		6		1	3		
		6	1				4
Text	261	17					1

N.B.: In compiling this table only those experiments were selected which were strictly comparable as regards dosage.

disease. To this point we shall return later for it is of capital importance as we shall see.

Those experiments of Neisser which are strictly comparable in so far as dosage is concerned, I have gathered together and placed in a single table (table 3) for the sake of comparison.

From a study of table 3 it is seen that, assuming that a successful reinoculation means cure and that a negative organ test means the same, there is complete agreement as to the results of treatment between the two methods of testing in 6 series, lack of complete

agreement in 5 and a question in the remaining 2 in which the results of reinoculation were equivocal. In justice to Neisser it should be stated that of those experiments in which the conditions were strictly comparable as regards dosage the total number of animals showing agreement between the two tests was 72, that showing failure of agreement 14, while the remaining 4 gave equivocal results.

Another and perhaps even more important question in relation to the experiments of Neisser which must be taken into consideration before accepting the validity of his contention that a successful reinoculation means the absence of the first infection and a failure to reinoculate means its presence, is the question of the time at which treatment was instituted in his experiments. The recent work of Kolle (46), Frei (40), Chesney and Kemp (49) and Adachi (64) has brought out very clearly the necessity of paying attention to this factor. The experiments of all these investigators, performed upon rabbits, are in complete accord and have established the fact that the time in the course of the disease at which treatment is begun is of prime importance in determining the character of the response of the host to a second inoculation. Thus rabbits treated before the forty-fifth day of the disease can be successfully infected a second time in almost every instance. If treatment is begun between the forty-fifth day and the ninetieth day of the disease the response of the animals to the second inoculation is variable in that some are reinfected and some are not. If treatment is postponed until after the ninetieth day of the disease the rabbits are almost universally refractory to a second inoculation. In the light of this now well-established fact it becomes necessary to reexamine Neisser's data obtained from monkeys and to determine in each instance the time at which treatment was begun and the subsequent response of the animal to reinoculation. Indeed this necessity is suggested by Neisser's own work, for, although he does not stress the point, his own experiments show that he obtained a considerably higher percentage of successful reinoculations in *untreated* apes when the second infection was introduced before the eightieth day of the disease than when it was introduced at a later date. If one arranges his reinoculation experiments in *untreated* monkeys according to the time at which the second inoculations were carried out, it is seen that the incidence of

positive results was 16.6 per cent when an interval of not more than 80 days was permitted to elapse between first and second inoculations. When, however, the second inoculation was carried out after the eightieth day of the disease only 3 positive results were obtained in 114 animals, an incidence of 2.6 per cent (table 4).

It is apparent that if undoubted second infections to the extent of 16.6 per cent are obtained in untreated monkeys when the second introduction of the virus is made before the eightieth day of the disease, whereas only 2.6 per cent of the animals can be reinfected after this period, then one might expect a higher percentage of successful reinoculations in monkeys treated early in the course of the disease than in those treated later, since it has been shown that in rabbits the time at which treatment is begun influences profoundly the response to

TABLE 4

Reinoculations in untreated apes (Neisser)

INTERVAL BETWEEN FIRST AND SECOND INOCULATION	TOTAL NUMBER	POSITIVE		NEGATIVE	
		Number	Per cent	Number	Per cent
days					
21–80	36	6	16.6	30	83.4
81–650	114	3	2.6	111	97.4

a second inoculation. It is, therefore, necessary to determine the time at which treatment was instituted in Neisser's experiments.

When one examines the protocols of his experiments in which treated monkeys were subjected to reinoculation, with reference to the time at which treatment was begun, one is disappointed to find that in many instances this information is lacking. I have been able to collect from his tables the records of only 18 animals in which the time elapsing between the first inoculation and the beginning of treatment was expressly stated or could be computed, although a total of 198 monkeys were treated and subsequently reinoculated in his therapeutic experiments. These I have arranged in tabular form (table 5).

Study of table 5 shows that, as a whole, the incidence of successful reinoculations in the animals treated by Neisser before the eightieth day of the disease (where this could be ascertained from the record) was 66 per cent, whereas in those treated after that date it amounted to

44 per cent. While the number of observations cited is admittedly too small to warrant any broad generalizations, especially in view of

TABLE 5

Reinoculation results in treated apes (Neisser)

TABLE	PAGE	ANIMAL NUMBER	INTERVAL BEFORE TREATMENT	REINOCULATION RESULT		DRUG
			days			
XXXVI	269	272	50	Negative		Phenylarsinic acid
		117	225	Positive		Phenylarsinic acid
		252	47	Positive		Phenylarsinic acid
XLIV	274	472	59	Positive		Arsenophenylglycin
		478	46	Positive		Arsenophenylglycin
		283	86	Negative		Arsenophenylglycin
			TREATMENT AFTER CHANCRE	FIRST REINOCULATION	SECOND REINOCULATION	
			days			
IL	299	597	129	Negative		Dioxydiamidoarsenobenzol
		606	106	Positive		Dioxydiamidoarsenobenaol
		608	113	Negative		Dioxydiamidoarsenobenzol
		619	2	Positive		Dioxydiamidoarsenobenzol
		622	5	Positive		Dioxydiamidoarsenobenzol
		624	8	Negative		Dioxydiamidoarsenobenzol
		567	223	Negative	Positive	Dioxydiamidoarsenobenzol
		568	223	Negative	Negative	Dioxydiamidoarsenobenzol
		604	171	Negative	Positive	Dioxydiamidoarsenobenzol
		616	4	Negative	Positive –	Dioxydiamidoarsenobenzol
		577	168	Doubtful	Negative	Dioxydiamidoarsenobenzol
		620	14	Doubtful	Negative	Dioxydiamidoarsenobenzol

Recapitulation

INTERVAL BETWEEN FIRST INOCULATION AND TREATMENT	TOTAL NUMBER	POSITIVE		NEGATIVE	
		Number	Per cent	Number	Per cent
days					
Less than 80	9	6	66.6	3	33.3
More than 80	9	4	44 4	5	55.6

the total number of animals studied, nevertheless their tendency is to indicate that in monkeys the earlier treatment is begun the more apt

is the animal to yield a positive result on reinoculation. Therefore it would seem highly desirable to take into account this factor of the amount of time elapsing between the original inoculation and the institution of treatment, before admitting that Neisser's results indicate that the reinoculation test is a valid criterion of cure. It is greatly to be regretted that the published protocols of Neisser fail to give the information which is necessary to clear up this question in so far as monkeys are concerned, and thus permit one to decide whether the criticism which is implied in the foregoing analysis is justified.

There is still another question which the recent work on reinfection in syphilitic rabbits makes it necessary to raise in regard to Neisser's reinfection experiments in monkeys, and that is the question of whether Neisser used homologous or heterologous strains for reinoculation. Kolle has demonstrated conclusively that rabbits infected with one strain and not treated may, in a large proportion of the cases, be successfully infected with another strain, even if the second infection is introduced comparatively late in the course of the disease. In some as yet unpublished experiments Chesney and Kemp have obtained similar results with heterologous strains in treated as well as untreated rabbits. It is by no means clear from Neisser's protocols whether he used homologous or heterologous strains in his reinoculation experiments in treated monkeys.

In the absence of data upon the points which we have raised, namely the time at which the animals were treated and the character of the strain used for reinoculation, it may perhaps seem wiser to some to consider that, in so far as apes are concerned, the contention of Neisser to the effect that in syphilis the acquired refractory state is dependent upon residual infection and disappears coincidentally with the elimination of that infection, has not been finally proved.

It would indeed seem rather strange, in view of what is known concerning the principles of immunity in general, if the resistance acquired as a result of syphilitic infection were sufficiently great to protect the animal against really large doses of a second infection, and yet vanish at once after the first infection had been eliminated. One would scarcely expect that such a profound change in the reacting capacity of the body toward new virus as is exhibited by the syphilitic animal, would disappear rapidly. Yet Neisser says that treated monkeys be-

come reinoculable immediately ("sofort"). Certainly one would not be justified in predicting such a result in syphilis on the basis of immune principles that obtain in most other infections.

If one takes the ground that this question of the dependence of immunity in syphilis upon the persistence of foci of infection has not been definitely settled by the earlier work upon monkeys, one may inquire as to what data bearing upon this point are available from studies of the infection in rabbits, and what data are afforded by the clinic with its opportunities for the observation of reinfections in man.

With regard to the first of these two questions, recent work, as stated previously, has clearly demonstrated that the time at which treatment is begun in the course of experimental syphilis of the rabbit has an exceedingly important bearing upon the response of the animal to a second inoculation. Kolle was the first to emphasize this point, although Brown and Pearce (65) had previously shown that treatment 18 days after inoculation with subcurative doses of arsphenamine or neoarsphenamine renders the rabbit susceptible to a second infection introduced 6 days later. Kolle demonstrated clearly that if syphilitic rabbits were treated with antisyphilitic drugs before the forty-fifth day of the disease they were almost uniformly susceptible to a second inoculation, whereas if the treatment were begun after the ninetieth day they were almost uniformly refractory to a second inoculation. If the treatment were begun at some time between the forty-fifth and the ninetieth day, some of the animals were refractory and some were not. The same results were obtained whether the treatment was short or prolonged.

Adhering to Neisser's views regarding immunity in syphilis and the validity of the reinoculation test as a criterion of cure, Kolle interpreted his own results as indicating that abortive cure of syphilis in the rabbit is possible with certainty only when treatment is begun comparatively early in the course of the disease, that is to say before the forty-fifth day, and impossible if it be postponed until after the ninetieth day. Frei obtained analogous results but was careful not to draw the conclusion that failure to reinoculate an animal meant failure to cure. He emphasized the importance of carrying out control observations with organs of treated animals but was unable to do so. Chesney and Kemp (48, 49) went a step further. They confirmed the obser-

vations of Kolle and of Frei to the effect that early treatment with arsphenamine leaves the animal susceptible to a second inoculation, whereas the late-treated animals remain refractory, but they showed in addition that the lymph nodes of these *same* animals were (through treatment) rendered incapable of transmitting the disease to normal rabbits, irrespective of the time at which treatment was begun. In other words, even if treatment were postponed until six months after inoculation, it was nevertheless apparently effective in sterilizing the lymph nodes and yet the *same animals* were subsequently shown to be refractory to a second inoculation. These observations suggested the possibility that in rabbits, at least, there may be such a thing as an acquired immunity resulting from syphilitic infection which persists in the absence of demonstrable infection, a view to which Adachi (64) subscribes.

Here again, the question of whether or not the treatment, when given late in the course of the disease, was effective in eliminating entirely the infection from the rabbit's body, constitutes the crux of the matter. Is lymph node sterility following treatment a certain index of cure? This is admittedly an exceedingly difficult question upon which to pronounce a definite opinion.[4] In a further series of experiments in which emulsions of lymph nodes and internal organs from untreated and late-treated rabbits were inoculated into normal animals, Chesney and Kemp (66) obtained evidence which suggested that the same treatment was effective in rendering those organs tested (heart, liver, spleen, bone-marrow, and brain), as well as the lymph nodes, non-infectious in a large majority of the animals. In all their experiments care was taken to maintain strictly comparable relations as regards dosage of drug and time of administration. The results of their experiments as to the efficacy of arsphenamine and its modifications in sterilizing lymph nodes were in strict accord with those obtained previously by Nichols and Walker (67) and also by Voegtlin, Armstrong and Dyer (68).

Chesney and Kemp (69) have obtained additional evidence which casts doubt upon the validity of the reinoculation method as a criter-

[4] A recent communication by Worms (160) shows how careful one must be in drawing conclusions as to cure, from the transfer of lymph nodes from treated animals to normal animals.

ion of cure. They found that while rabbits, which were originally inoculated in the testis and treated late in the course of the disease, were almost uniformly refractory to a second intratesticular inoculation, other syphilitic rabbits similarly treated and reinoculated by a different method (deposition of virus upon a granulating wound on the back) were in 50 per cent of the cases susceptible to a second infection. In other words, the mode of reinoculation in treated animals as well as the time at which treatment was begun was found to play an important rôle in determining the response of the animal to a second inoculation. If markedly divergent results from reinoculation are obtained in otherwise comparable groups, depending upon the particular method selected for reinoculation, it is at once apparent that no conclusion can be drawn, as to the presence or absence of the first infection, from the response of an animal to reinoculation.

It would appear from the recent work with rabbits therefore, that the validity of the reinoculation test as a criterion of cure of syphilis in the rabbit has definitely been brought into question, a statement that is supported by a recent communication from Voegtlin's laboratory (70). It necessarily follows that the contention of Neisser as to the inter-dependence of syphilitic immunity and active syphilitic infection is likewise brought into question. It may not be amiss to point out that as long ago as 1865 Rollet (11) took the stand that immunity to syphilis does not necessarily imply the existence of syphilitic infection, and many years later Finger (71) expressed the same idea. Indeed Neisser's contention has not been without its critics (72, 73) even in recent years, although in general his idea has been almost universally accepted by syphilologists and immunologists as well.

As suggested previously it may be of interest to ascertain what clinical observation has to contribute to the question. That second attacks of syphilis do occur has long been known, but the number of such cases has been extremely small. This relative infrequency of second attacks of syphilis was thought by Neisser to be due, not to a true immunity surviving after the infection had been eliminated, but to two other factors: first, that the number of uncured cases of syphilis is greater than was formerly realized, a contention which the data supplied by the Wassermann reaction would seem to uphold; secondly, that there is less exposure to infection with advancing years

of life by reason of changing social relationships, especially that of marriage. Whether these two factors together would be sufficient to account for the great infrequency of second attacks of syphilis is, and probably always will be, a matter of speculation. But it is also possible that this infrequency may in part be explained upon the basis of an acquired immunity persisting throughout life, even after total eradication of the infection by suitable treatment. Clinical experience in respect of reinfections is certainly not in conflict with this conception.

There seems to be general agreement that the number of undoubted instances of reinfection is greater now than before the introduction of salvarsan. This fact has been urged by some as an index of the greater efficacy of this drug over older forms of treatment, and has also been utilized by those who adhere to Neisser's view of the interrelationship between persistent infection and immunity. Briefly their argument is as follows: More cases of reinfection are seen now than formerly because more patients are cured and hence become susceptible again to syphilis. It must be obvious that this argument does not dispose of the conception of acquired immunity to syphilis persisting in the absence of infection, since it is conceivable that in many cases treatment with salvarsan might be, and doubtless is, postponed until a considerable time interval has elapsed after infection, during which interval a considerable degree of resistance may have been acquired by the host, and yet the latter might nevertheless have been cured. In other words, in man, as in the rabbit, the time at which the first infection comes under treatment may be the all-important factor in determining the response to a second infection, and *ipso facto* the number of cases of reinfection which will be encountered in the clinic. In view of this possibility it becomes important to ascertain the time at which treatment was first begun in the cases of reinfection that are recorded in the literature. If one could assemble a large number of cases of undoubted reinfection in which the patient had not received treatment for his first infection until a comparatively late date in the course of his disease, say the latent period following the healing of the secondary lesions or the tertiary period, one would have to admit that the case for the existence of syphilitic immunity in man in the absence of syphilitic infection would be greatly weakened if

not altogether demolished. Actually, however, no such array of cases has as yet been assembled.

By far the overwhelming majority of cases of undoubted reinfection that have been reported thus far have occurred in patients in whom treatment was begun early in the course of the disease. The undoubted instances in which a patient was treated for the first time late in the course of his infection and subsequently contracted syphilis a second time are exceedingly few in number, although by now the literature contains many examples of undoubted reinfections. Benario (74) in 1914 was able to gather together from the literature of 1910–1914, 96 cases of undoubted syphilitic reinfection. These cases were rigidly selected. All of these patients had been treated with salvarsan alone or in combination with mercury. Patients treated with mercury alone were not included in his report. In all but one of these the treatment for the first infection was begun during the early stages of the disease. In the one exception the first infection dated back 23 years prior to treatment with salvarsan. At the time this particular patient received his first dose of salvarsan there were no physical signs of syphilis but the Wassermann reaction was positive. One may object to this evidence upon the ground that at the time Benario collected his cases there had not yet been opportunity for a sufficiently large number of patients with late syphilis to have been treated with salvarsan and become reinfected, and that the number of early cases treated up to that time was far in excess of the number of late cases treated. There are, of course, no data on this point, but a survey of the cases of reinfection reported in the literature since 1914 indicates essentially the same situation disclosed by Benario. Dr. H. Wasserman has, at my suggestion, undertaken from this standpoint a survey of the cases of reinfection reported in the literature since the appearance of Benario's communication and also the reinfections encountered in the syphilis clinic of the Johns Hopkins Hospital. While that survey is not as yet complete, it has progressed sufficiently far to show that practically all the cases of undoubted reinfection have occurred in individuals who received treatment for their first infection early in the course of their disease.

Clinical experience in respect of syphilitic reinfection in man, therefore, does not controvert the idea that in this infection an immunity

may develop in time which will persist after the infection has been abolished. It is obvious, however, that the same experience may be interpreted as indicating that cure of syphilis in human beings is possible only when treatment is begun early in the course of the disease and not at a later date. At present it is not possible to say, from the data available from human sources, which interpretation is the correct one.

The question of the development in human beings of a true immunity to syphilis has recently been discussed by Kolle (51) from a new angle. Upon what might perhaps be called epidemiological grounds he seems to have come to the conclusion that human beings do acquire a true immunity during the course of an attack of syphilis. His conclusion is based upon the following argument. According to him, sterilization of the syphilitic individual by means of antisyphilitic agents, especially salvarsan in combination with mercury, is possible in a large proportion of the cases when treatment is begun early. If immunity of human beings to syphilis is dependent only upon latent infection, then many cases of reinfection ought to be encountered since modern treatment of the early syphilitic often leads to complete cure, according to Kolle. Granted that in Germany, during the course of a year, only 10 to 20 per cent of persons with early syphilis receive abortive treatment and hence are cured, then in the course of time one should see in that country many thousands of cases of reinfection occurring in cured individuals who cannot, because of cure, be considered as having a latent infection and an immunity dependent thereon. Actually, however, one does not see any great number of such cases of reinfection. They are in reality very scarce. Kolle, therefore, concludes that this scarcity is dependent upon an acquired immunity which develops in the syphilitic during the course of his infection and persists after a mode of treatment which he thinks effectually eliminates the disease.

It is obvious that the validity of Kolle's conclusion rests upon the truth of two premises, one of which is expressed, the other implied; first, that early treatment of syphilitic human beings with the modern means of attack does result in biologic cure in a large proportion of cases, second, that those individuals who have been infected and are promptly and adequately treated continue to be exposed to infection

after treatment has ceased, and indeed that exposure occurs about as frequently as before infection. Most syphilologists will probably assent to the first of these two propositions, namely that syphilis in the human being can be cured with early and systematic modern treatment. As regards the truth of the second proposition, that individuals who have once contracted syphilis and been treated for it continue to be exposed to the same infection to something like the extent obtaining prior to their having contracted the disease, that question must necessarily be a matter of conjecture since we are not likely ever to have any accurate data upon it. Nevertheless, Kolle's argument implies that such continued exposure does actually take place in Germany, and there is every reason to believe that the same state of affairs obtains for that portion of the American population in which syphilis is most prevalent. Certainly Kolle's argument is unique, suggestive and rather convincing.

Kolle concludes from the same train of reasoning that the immunity which the human being acquires as a result of his infection is effective against heterologous strains as well as the homologous strain, and he regards it as of a higher or different order than that which rabbits acquire and which for the most part is effective against homologous strains only. Before placing relative values upon the resistance which develops in man and that which develops in the lower animals in the course of syphilitic infection, it may be well to recall that doses of virus are used in performing reinoculation experiments upon the latter which may exceed any that human beings are apt to encounter; furthermore, the mode of inoculation is quite different, and in the experimental infection certainly favors the invading microörganism rather than the host. In short, apparent differences in degree of acquired immunity to syphilis as seen in man on the one hand, and in the monkey and the rabbit on the other, may possibly be resolved upon the basis of the size of the inoculum of the second infection, or the mode of its introduction. Further studies on the quantitative relationships of this problem are highly desirable.

It may be of interest at this point to call attention, in passing, to a recent communication by Prigge (75) from Kolle's laboratory, in which support is advanced for the view that the immunity which mice, that have recovered from relapsing fever, exhibit toward a second in-

fection, is a true immunity, and is not dependent upon residual foci of infection in the brains of these animals, a view that had been proposed by Buschke and Kroo (76).

Summary. The conception that acquired immunity in syphilis is dependent upon foci of syphilitic infection somewhere in the body was first advanced by Neisser upon the basis of experiments on monkeys. Analysis of his experiments in the light of recent contributions to this subject has raised the question as to whether or not his conception is justified. More recent work based upon experiments on rabbits would seem at present to controvert this view and to indicate that in syphilitic infection there may develop an immunity which is not dependent upon the persistence of foci of infection. Clinical experience in respect of reinfections in man is entirely in harmony with such a conception. The whole question is in intimate relation with that of the curability of syphilis, a problem which is most difficult of solution when the term cure is used in the biological sense. Perhaps it may be well for the present to take the ground that this particular phase of the problem of immunity in syphilis is still an open question, but it must be admitted that evidence is now being accumulated which would indicate that during the course of syphilitic infection the host acquires a resistance against a second infection with the same species of microörganism, a resistance which may persist after the first infection has been eliminated by treatment, or at any rate reduced to the level where it cannot be detected. If the truth of this proposition becomes firmly established, it will follow that reinoculation tests in animals or the occurrence of reinfections in man have no bearing upon the question of persistence or absence of infection, that is to say of cure or failure of cure, but are useful only as indicators of specific resistance. Logically speaking, in any case all that one can justifiably conclude from failure to reinfect an animal with syphilis is, that the animal is resistant to that infection.

Criteria of reinfection

In almost all of the experiments dealing with syphilitic reinfection in animals the criterion of a successful reinoculation has been the production of a syphiloma at the site of reinoculation accompanied by the demonstration of treponemes in the lesion. This is, of course,

the safest criterion. In dealing with reinfections in man it has generally been considered necessary to go a step further and demand that the lesion produced at the portal of entry be followed by evidences of systemic infection. No one can object to the utilization of the most rigid criteria when dealing with possible reinfections in man. However, since the tendency of syphilis to pursue a variable course, even during its early phases, is well known, and since syphilitic infections are known to occur in man without any lesion at the portal of entry and such infections can be produced in certain of the lower animals, it is theoretically possible that reinfections might pursue a similar course, nay more, there might be an even greater tendency for reinfections to manifest themselves in such a manner. Neisser himself admitted this possibility but stated that he had never observed an example of it. Brown and Pearce (77) called attention to this matter several years ago and pointed out that the development of a chancre in a reinoculated animal "is of more value as an index of the ability to produce a manifestation of disease than of infection, and that infection can not be excluded upon this basis." They went on to say: "It would appear, therefore, that before the results of super-inoculation experiments can be made clear, the subject must be approached from a broader point of view and that evidence must be adduced which will enable one to see beyond the reaction at the site of inoculation."

This problem lends itself to study better in treated than in untreated animals. Chesney and Kemp (69) have assembled evidence which indicates that it is possible to reinfect a treated rabbit without producing any lesion whatsoever at the site of reinoculation. Nevertheless, in the hands of these investigators such animals give evidence of systemic invasion by the treponemes as evidenced by lymph node transfer, and in a large proportion of the cases a positive Wassermann reaction appears in the blood. It would seem from their work that at least two factors may be involved in bringing about such an altered response to a second inoculation; these are (1) the time at which treatment is begun, and (2) the manner in which the second inoculation is made. It is not inconceivable that the richness of the inoculum in treponemes or the virulence of the organisms may also constitute factors in producing such a response but as yet there are no data upon these points. Voegtlin and Dyer (70) have also obtained results in

syphilitic rabbits treated with arsphenamine or sulpharsphenamine and then reinoculated with the same strain of treponemes, which lend support to the view that reinfections may occur without local lesions. More recently Kolle (78) has raised the same question and has indicated that he possesses evidence, which as yet has not been published, to the effect that second infections can be produced without the appearance of any local lesion at the portal of entry.

On the basis of their observations Chesney and Kemp have suggested that some of the relapsing Wassermann reactions occurring in patients with early syphilis who have been well treated may represent not a relapse of their first infection, as is generally held, but a true reinfection in which no lesion occurs at the portal of entry but in which systemic distribution of the virus takes place and is accompanied by the reappearance of a positive Wassermann reaction. In other words, a second infection may have taken place in such individuals and have undergone an unusual course by reason of the fact that it was introduced into a patient still under the influence of an immunologic change brought about by the first infection. Such individuals, if they exist, might be regarded as partially refractory to a second infection. It must be admitted that at present in clinical practice we have no means of differentiating such patients from those in whom the subsequent re-appearance of a positive Wassermann reaction represents a true serological relapse but the future may supply us with the means. For the present, however, in dealing with human beings, it would seem best to confine ourselves to the older and safer criteria of reinfection and demand a characteristic primary lesion followed by the evolution of secondary manifestations. In the experimental animal, however, it is probably not necessary to demand visible macroscopic lesions at the site of reinoculation before concluding that the animal has been reinfected, for in the case of the latter there are available methods, not applicable to the patient, of determining that infection and dissemination of treponemes has occurred.

ACTIVE IMMUNITY ARTIFICIALLY ACQUIRED

Active immunization as a therapeutic measure in syphilis was attempted in France as early as the first half of the last century. Doubtless the success attending small-pox vaccination was largely

responsible for the attempt. Auzias-Turenne (79) conceived the idea of performing successive auto-inoculations upon patients with chancres in order to prevent the occurrence of secondary lesions and to this procedure he gave the name of "syphilization." He thought he was successful in preventing constitutional syphilis in this way but it is clear that he must have been working with material in which pyogenic organisms were present and his results failed of confirmation by others. Nevertheless, a rather widespread and bitter controversy arose in France over the merits of the method but it quickly fell into disrepute and is of historical interest only at the present time.

The recognition of the causal agent of syphilis and the transmission of the infection to animals gave further impetus to the attempts to immunize against the infection but, although a considerable number of experiments have been performed, the results have thus far been disappointing. In presenting this phase of the subject we shall find it profitable to approach it from the standpoint of the nature of the material used for immunization.

Filtrates and extracts. De Luca and Casagrandi (80) injected 6 human volunteers intramuscularly with filtrates of primary lesions. Two of these individuals later contracted syphilis after exposure to infected persons, although the remaining 4, in spite of opportunity for infection, remained free of symptoms while under observation. Unfortunately, the period of observation was relatively short (2 months). The extracts had no therapeutic effect on patients with secondary lesions. Metschnikoff and Roux (81) injected a chimpanzee with the filtrate from several human syphilitic lesions diluted with the aqueous humor of a sheep but were unable to obtain any evidence of immunity in the animal since a subsequent inoculation with non-filtered virus produced disease. Neisser extracted chancres, condylomata and the organs of syphilitic fetuses with normal salt solution containing 0.5 per cent phenol and attempted to immunize apes with such extracts but obtained no evidence of immunity whatsoever. Even when the test virus was inoculated at the same spot where the immunizing extract had been injected, a lesion made its appearance, indicating the powerlessness of such extracts to confer even a local immunity. Furthermore, injections of these extracts carried out during the incubation period failed to prevent the development of syphilitic lesions.

Truffi (82) injected rabbits with extracts prepared from dried pulverized livers of syphilitic fetuses in an attempt to immunize these animals against syphilitic infection but was unsuccessful. It is possible to say, therefore, that various manipulations of syphilitic tissue containing treponemes have not thus far yielded substances with any appreciable antigenic power so far as preventing or modifying the course of syphilis is concerned.

Killed virus. Metschnikoff and Roux (81) inoculated a chimpanzee with virus from human sources killed by heating to 51°C. for an hour. A subsequent inoculation of the same animal with active virus resulted in the development of a characteristic syphilitic lesion, indicating that heat-killed virus has no immunizing power in this species of animal. Finger and Landsteiner attempted to immunize 3 monkeys with subcutaneous injections of heat-killed virus but were unsuccessful in each instance. Equally fruitless were the attempts of Uhlenhuth and Mulzer to protect rabbits against syphilitic infection by the subcutaneous and intravenous injection of syphilitic virus killed by phenol or antiformin. They found also that a vaccine prepared from the testis of a syphilitic rabbit by drying the finely divided tissue on a glass plate and then suspending it in salt solution was equally ineffective in protecting rabbits against a subsequent inoculation with living syphilitic virus. Such vaccines when administered to rabbits already inoculated with syphilitic virus failed to exert any effect upon the course of the disease.

Living virus. Of interest are the attempts of Spitzer to prevent the development of generalized lesions of syphilis in man by active immunization with living virus from patients presenting primary lesions. This procedure was undertaken at the suggestion of Kraus who thought that he saw a resemblance between rabies and syphilis in that in both diseases, as he supposed, a comparatively long interval elapsed between infection and generalization of the virus. Accordingly Kraus concluded that immunization of human beings with syphilis as soon as the diagnosis could be made and before the outbreak of secondary lesions, utilizing living virus, might prevent the occurrence of constitutional symptoms just as immunization of human beings with rabies during the incubation period often prevents the development of symptoms of that disease. The hypothesis was put to the test by

Spitzer (83) who treated a series of syphilitic patients with injections of emulsions of human chancres, using 2 cc. of 1:200 dilution and increasing the concentration to 1:40. From 11 to 20 injections were given and in a series of 20 patients Spitzer claimed that secondary lesions failed to appear in 7 instances (35 per cent), although the patients were under supervision for a period of many months, the maximum period of observation being 2 years. One of the patients was thought later to have contracted a second attack of syphilis, a significant observation if true. The method was tried by Brandweiner (84) and also by Kreibich (85), both of whom reported unfavorable results. As Neisser has pointed out, in criticising this form of treatment upon theoretical grounds, generalization of the infection has already taken place by the time the chancre has made its appearance and the treponemes are being distributed all over the body by the circulation. Under these circumstances it seems unlikely that the subcutaneous injection of additional syphilitic virus in comparatively small amounts would hasten the development of the immune reaction of the host or accentuate it. Similar treatment of apes by Neisser, as we shall see presently, proved to be wholly ineffective, and this fact alone would make one extremely skeptical of the value of the procedure in man. Still, as Zinsser says, it is the only ray of light in what is otherwise a rather dark situation, but perhaps it is not a very bright ray.

Metschnikoff and Roux (86) sought to attenuate the virus of syphilis by passage through lower apes with the intention of utilizing such virus for immunization purposes. Indeed they came to the conclusion that they had been able to secure attenuation to such an extent that the virus was capable of producing in rhesus monkeys a comparatively insignificant lesion at the portal of entry which was not followed by any evidence of generalization of the infection. Nevertheless, the monkeys were refractory to a second inoculation. They thought, by an experiment upon a human volunteer, that they had secured attenuation of the virus for the human being as well. Neisser criticized their experiments rather vigorously in the light of his own work and suggested that Metschnikoff and Roux had not appreciated the fact that in the monkey, infection and generalization of the disease could occur although the lesion at the site of inoculation might be small or even non-existent. Neisser himself was unable to obtain the

slightest evidence of attenuation of syphilitic virus, either by passing it through monkeys or by treating it with chemical agents which themselves were not treponemicidal.[5]

Finger and Landsteiner inoculated 2 monkeys with living virus, introducing the material deep into the muscles and then cauterizing the needle tract. No syphilitic lesion developed along the tract in either animal. Subsequent inoculation with living virus by scarification of the skin gave a positive result in one of the animals and a negative result in the other. Three monkeys inoculated intraperitoneally by them before or at the time of a cutaneous inoculation proved refractory to the latter. Neisser in a large series of experiments on monkeys found that the subcutaneous or intravenous injection of living virus at the time of the first (cutaneous) inoculation failed absolutely to prevent the development of a primary lesion at the site of the latter; furthermore, the same methods employed after the primary lesion had developed failed to prevent dissemination of the virus throughout the body.

In the hands of Uhlenhuth and Mulzer intravenous inoculation of living syphilitic virus failed to protect rabbits against intratesticular inoculations carried out 18 and 35 days later. Subcutaneous injection of living virus also failed to protect against intratesticular inoculation 50 days later.

Cultures. It was natural that the successful cultivation of *Treponema pallidum* from human lesions should be followed by attempts to immunize animals with these cultures and to approach the study of immunity in syphilis by their aid. However, in spite of the large amount of time and effort expended, the results have in the main been disappointing. Nakano (89) gave 3 patients with early syphilis subcutaneous injections of killed cultures of treponemes but was unable to observe any influence upon the course of the disease in these patients. In his hands rabbits injected intravenously with cultures of treponemes exhibited agglutinins in their serum, the maximum titer reached being 1:60, nevertheless some of the immunized animals

[5] The occurrence of syphilis in laboratory workers, brought about by accidental infection with virus that had been carried through many generations of animals does not support the view that attenuation of syphilitic virus for man is accomplished by passage through other animal species (87, 88).

proved later to be susceptible to intratesticular inoculation with virus from rabbit testes in spite of the presence of agglutinins for treponemes in their blood. Zinsser, Hopkins and McBurney (90) succeeding in producing agglutinating sera of high titer (1:2000 to 1:4000) by intravenous immunization of rabbits with cultures of treponemes but, like Nakano, found that their animals were still susceptible to intratesticular inoculation with virulent syphilitic virus. They found also that intratesticular inoculation of rabbits with living cultures of treponemes failed to protect the animals against subsequent (intratesticular) inoculation of virulent treponemes obtained from syphilitic rabbit lesions.

Grouven (91) treated 2 syphilitic rabbits with Sowade's mixed cultures of treponemes and thought he observed a favorable effect upon the syphilitic lesions of both animals as evidenced by rapid healing. He likened the effect to the focal reaction observed at times after the injection of tuberculin. In spite of this apparently favorable result one of the animals subsequently exhibited recurrent lesions and it seems doubtful if the treatment had any real effect upon the course of the infection. In the hands of Schereschewsky (92) the injection of killed cultures of treponemes treated with antiformin appeared to protect 2 rhesus monkeys against a subsequent inoculation with virulent syphilitic virus from human sources but the injection of a culture containing living treponemes failed to protect a third monkey against the same virus. It should be noted that in these experiments the animals were observed for only six weeks after inoculation, a rather short time in experiments of this sort. Noguchi (93) thought that repeated intratesticular injections of rabbits with cultures of treponemes or killed virus from lesions reduced their susceptibility to syphilitic infection "to some extent," but since at least half of the animals thus treated were successfully inoculated later with virulent syphilitic virus the degree of resistance established by this method of immunization cannot be regarded as of a high order.

Sagel (62) has recently reported upon a series of 10 paretics treated with subcutaneous and intracutaneous injections of living treponemes (cultures or rabbit virus), also by scarification with the virus. His idea was that perhaps by bringing living syphilitic virus in contact with the skin a greater immune response to the infection might be

elicited and a favorable effect exerted upon the course of the disease process in the brain. The patients selected for this method of treatment had not shown any improvement following the production of artificial relapsing fever and they were regarded as having a hopeless prognosis. Of the 10 patients, 7 showed clinical and serological improvement, 1 showed serological improvement only and the remaining 2 showed no improvement. It is obvious that these patients have not been followed for a sufficiently long period of time to determine the final outcome and no judgment as to the method can be formed as yet. In none of the patients were syphilitic lesions produced at the site of inoculation and the method would certainly appear to be harmless.

Summary. From the foregoing it is clear that there is general agreement among all the workers who have attempted to produce active immunity to syphilis in man, monkeys or rabbits. The almost universal experience has been that of failure, no matter what kind of material has been employed as antigen. Extracts of tissue containing treponemes, killed virus, living virus, cultures of treponemes, all as a rule have proved incapable of rendering the animal immune to a subsequent inoculation with virulent organisms. The experiments of Schereschewsky and of Noguchi are too few in number to invalidate this statement. In the few instances in which apparently successful results have been reported after the use of living "attenuated" virus it is by no means certain that the investigators had excluded the possibility that a general infection had been established and that the apparent immunity which they observed was the result of actual infection which had been overlooked. Nor has it proved possible to alter the course of an existing syphilitic infection by immunization with any product derived from cultures of treponemes or from syphilitic tissue. While it is true that Spitzer claimed to have modified the course of syphilis in human beings by active immunization during the early period of the disease with living virus from human sources, and favorable results have been reported by one observer who treated a small series of paretics with living syphilitic virus, the observations of these workers have not been confirmed and the method has not as yet found any recognized position in the treatment of syphilis.

PASSIVE IMMUNIZATION

If the manifold attempts actively to immunize man and animals against syphilis by the introduction of virus or virus-products have thus far been destitute of any significant results, it might be expected that the serum of such animals would fail to exert any protective or curative action in the natural or experimental disease. Unhappily the facts bear out that expectation. Attempts to bring about passive immunization against syphilis were made long before the causal agent of the disease had been discovered or the infection had been transmitted to lower animals. A curative serum was sought in human beings that had had the disease as well as in animals inoculated with syphilitic tissue. The earlier work has been summarized by Neisser (94) and it is unnecessary to discuss it here in any detail. Some of the procedures attempted in the past appear now as very bizarre. In spite of the extravagant claims of some workers the ultimate results of all this work were valueless. With the successful transmission of syphilis to lower animals interest was renewed in the subject since the production of an experimental infection gave an opportunity for more accurate evaluation of any serum that might be produced.

Finger and Landsteiner treated 2 patients presenting chancres, with serum from syphilitic apes, having first excised the chancre in one case and both chancre and inguinal nodes in the other. In spite of this treatment secondary manifestations developed in these patients in the usual manner. Neisser carried out a large series of experiments which had for their purpose the demonstration of the possibility of passive immunization against syphilis. It may be said at once that they all yielded negative results. He inoculated horses, cattle, sheep, and monkeys with both living and killed virus from both human and animal sources and then tested the serum of these animals as regards (1) its treponemicidal power *in vitro*, (2) its protective power *in vivo* in apes when injected locally at the site of inoculation and when administered subcutaneously or intravenously, and (3) its curative power in apes when given intravenously or subcutaneously after infection had been established. In no instance was there any evidence that the serum exerted any effect upon the course of the disease or modified the virus *in vitro*.

The results in rabbits have been equally fruitless. Truffi (82) attempted to protect rabbits against syphilitic infection by treating them with serum from other rabbits that had proved to be immune to a second infection with syphilitic virus. In spite of treatment prior to inoculation the test animals proved to be as susceptible as normal animals to virus introduced into the subscrotal tissue. Uhlenhuth and Mulzer found that the serum of rabbits that had received several injections of living syphilitic virus was totally incapable of modifying the course of syphilis in other rabbits either when given at the time of inoculation or after the appearance of clinical manifestations of infection. Moreover, such serum was incapable of agglutinating treponemes or of forming a precipitate when brought into contact with an emulsion of these organisms. Nakano (89) injected rabbits intravenously with cultures of treponemes and found that the serum of these animals, although it possessed some agglutinating power for the organisms in the culture, had no protective power *in vivo* and failed to exert any beneficial action when administered to syphilitic rabbits, either intravenously or locally at the site of the syphilitic process.[6]

The question as to whether or not immunity to syphilis can be transmitted to offspring deserves consideration at this point. For a time it was thought that such might be the case and, as is well known, the principle was expressed in the form of a law (Profeta's law), to the effect that the offspring of a syphilitic woman might nurse an infected woman with impunity and never show signs of syphilis. The advent of the Wassermann reaction served to show that these supposedly immune children were in reality themselves syphilitic and that their immunity was presumably dependent upon their own infection and not upon a passive transfer of immune bodies from the mother. Nobody has yet succeeded in bringing forward any convincing evidence that the offspring of syphilitic parents are immune to syphilis and at the same time entirely free of syphilitic infection. Nevertheless, the idea has been entertained in some quarters that the refractory state which develops in syphilitic individuals may in

[6] Jáuregui and Lanceotti (161, 162) have reported that they have obtained an immune serum from lamas which, when administered to animals of this species infected with syphilis or to syphilitic patients, exerts a marked beneficial effect upon the course of the disease.

part be transmitted from parent to child, and that in this way in the course of time the human race may gradually build up a heightened resistance to the disease. Some of those who have been attracted to this hypothesis have contended, in support of it, that the course of syphilis is milder now than it was several centuries ago. Neisser was unwilling to accept such a conclusion, however. He thought that the milder course of syphilis nowadays may be referable to a number of other factors, such as more precise differentiation of syphilis from other diseases, better individual hygiene, and better therapeutics. Moreover, he pointed out that when syphilis makes its appearance in virgin soil, so to speak, it does not always pursue a malignant course. Furthermore, in Neisser's opinion, in lands where the disease has been prevalent for years the course of the disease is not appreciably different from that in countries where it is not so prevalent. On the whole, then, there is little or no evidence to show that acquired resistance to syphilitic infection can be transmitted through inheritance, just as there is at present no satisfactory evidence to show that passive immunization can be accomplished or the course of an established infection altered by the administration of serum from immune persons or animals.

ANTIBODIES IN SYPHILIS

Attempts have naturally been made to demonstrate in the blood serum of syphilitic patients or of animals that have been experimentally infected with syphilis, the presence of antibodies analogous to those that have been found in other infections. The reports that have thus far appeared have yielded rather divergent results so that the matter is in a somewhat confused state at the present time.

Agglutinins. a. Man. Numerous attempts have been made to demonstrate agglutinins for *T. pallidum* in the serum of patients with syphilis. Hoffman and v. Prowazek (95) exposed emulsions of treponemes to the action of serum from patients with untreated syphilis of 6 to 8 months duration and thought they observed some tendency on the part of the organisms to undergo agglomeration and loss of motility. They expressed themselves in a very conservative fashion, however, concerning this phenomenon and one gets the idea that it was not a particularly striking one. Zabolotny and Maslakowetz

(96) obtained analogous results but Landsteiner and Mucha (97) failed to obtain any evidence of agglutinating power on the part of the serum of patients with syphilis. These investigators, however, observed a tendency toward agglutination in preparations of material from human syphilitic lesions and suggested that this phenomenon might be due to the formation of agglutinins locally at the site of the syphilitic process although no such agglutinins were observed in the serum of the same patients. Uhlenhuth and Mulzer (98) were unsuccessful in demonstrating in the serum of patients with syphilis, agglutinins for treponemes derived from syphilitic lesions. On the other hand, Touraine (99), using the microscopic method, concluded that he was able to demonstrate agglutinins for tissue treponemes in the serum of a number of syphilitics with varying manifestations of the disease, and was of the opinion that the procedure might be utilized for the diagnosis of syphilis. Kissmeyer (100) found that the serum of some syphilitic patients agglutinated cultures of treponemes in higher dilution than did the serum of normal persons and Kolmer, Broadwell and Matsunami (101) had essentially the same experience. On the other hand, Arnheim (102) obtained negative results and Zinsser, Hopkins and McBurney (103) found that the serum of syphilitic patients did not agglutinate cultures of treponemes in an appreciably higher dilution than did normal human serum, and did not agglutinate treponemes obtained from syphilitic lesions. In their opinion agglutination could not be employed as a diagnostic measure. Siemens and Blum (104) extracted the skin of paretics and sought to demonstrate the presence of treponeme-agglutinating substances in these extracts but were unsuccessful.

b. Rabbit. Uhlenhuth and Mulzer (98) were unable to obtain any evidence of agglutinins in the serum of rabbits experimentally infected with syphilis. On the other hand, Zinsser, Hopkins and McBurney (103) found that the serum of syphilitic rabbits agglutinated cultures of treponemes in dilutions of 1:25 to 1:50 but did not exert any agglutinating or immobilizing effect upon virulent treponemes from rabbit lesions. Blum (105) also worked with suspensions of treponemes obtained from syphilitic lesions in the rabbit and found that the serum of syphilitic rabbits agglutinated these organisms at times in dilutions as high as 1:100, while only occasionally did normal rabbit serum

agglutinate them and never in dilutions greater than 1:10. In his experience the occurrence of agglutinins seemed to be dependent upon the duration of the infection and the extent of the clinical manifestations. Almost all workers in this field have reported the production of agglutinins in the serum of rabbits after the intravenous injection of cultures of these organisms, the maximum titer varying with different observers: [Nakano, 1:60 (89); Kolmer, 1:1280 (106); Zinsser and Hopkins, 1:4000 (107); Kissmeyer, 1:20,000 (100); Noguchi and Akatsu, 0.000025 cc. (108)]. Zinsser, Hopkins, and McBurney (109) made the important observation that a serum which would agglutinate culture treponemes in a dilution of 1:4000 would not agglutinate the same strain when the organisms were obtained from syphilitic lesions in the rabbit. For some unexplained reason the virulent organisms could not be affected by the agglutinating serum, although the culture treponemes were readily agglutinable. By means of cross agglutination and absorption experiments these investigators showed that treponemes from different sources were "related in group reactions."

Treponemicidal substances. Finger and Landsteiner inoculated a series of 12 apes with mixtures of virus and serum from patients in various stages of syphilis, the serum-virus mixture being allowed to stand for $\frac{1}{2}$ hour before inoculation. In each animal a lesion developed at the site of inoculation indicating that the serum did not possess a high degree of treponemicidal activity. Nakano (89) was unable to demonstrate the presence of treponemicidal substances in the serum of rabbits immunized with cultures of treponemes when he exposed the treponemes to the action of the serum in the test tube. When he injected the immune serum and treponemes into the peritoneal cavity of guinea pigs, however, lysis of the organisms took place promptly. Control inoculations with normal rabbit serum under the same conditions did not bring about lysis nor did the serum of patients with early or late syphilis. So far as the writer is aware Nakano is the only investigator who has reported the occurrence of the Pfeiffer phenomenon in association with the serum of animals immunized with *Treponema pallidum.*

Zinsser and Hopkins (110) found that the serum of rabbits and of sheep immunized with cultures of treponemes exerted a treponemici-

dal action upon cultures of treponemes *in vitro*. According to them the treponemicidal activity was abolished by heating at 56°C., but could be reëstablished by the addition of fresh normal serum. This observation would, of course, bring the treponemicidal substance or substances into line with other well known antibodies. According to these investigators the property was not strain specific and normal serum was found by them to possess a slight degree of treponemicidal activity although by no means to the same extent as did the serum of immunized animals. Later Zinsser, Hopkins and McBurney (109) showed that the treponemicial activity of the serum of the immunized animals was not effective against virulent treponemes derived from syphilitic lesions in the rabbit, although it retained its activity for culture treponemes. Noguchi and Akatsu (108) succeeded in demonstrating the presence of treponemicidal substances in the serum of rabbits immunized with cultures by intravenous injections repeated over many weeks. In some instances amounts of serum as small as 0.0003 cc. were, in their hands, capable of destroying the treponemes in the test tube. The addition of complement was found to enhance the treponemicidal activity appreciably. Microscopical examination of the contents of the tubes in which the reaction took place revealed striking changes in the morphology of the treponemes. The serum of a syphilitic rabbit was found to exhibit treponemicidal activity in amounts greater than 0.03 cc., whereas 0.1 cc. of normal rabbit serum exerted only slight restraint of growth.

Eberson (111) investigated the treponemicidal activity of serum from human beings in various stages of syphilis and found no evidence of such in 7 patients with recent infections. On the other hand, the serum of 18 patients with latent syphilis in whom the infection was thought to have occurred at a more remote time was found to possess treponemicidal power for virulent treponemes derived from rabbit lesions. In his experiments the serum was allowed to act upon the virus in the test tube, the mixture being subsequently inoculated intratesticularly into rabbits. Treponemicidal activity did not go hand in hand with a positive Wassermann reaction since it was observed in several specimens of serum obtained from treated patients whose Wassermann reaction was negative. The serum of syphilitic rabbits was found to possess treponemicidal power when the animal from

which the blood was obtained had had its infection for 6 months or more, but treponemicidal activity could not be demonstrated in the serum of rabbits whose infection was of shorter duration. It should be noted that in Eberson's experiments the serum was preserved for some time and was heated at 54°C. for 20 minutes before testing; also that he did not apparently find it necessary, as had Zinsser and his coworkers, to add complement in order to bring about the reaction. It is, of course, conceivable that the small amount of serum or tissue juice in which the treponemes were suspended was sufficient to supply any complement that may have been necessary to ensure the success of the reaction. So far as the writer is aware these experiments are the first in which treponemicidal activity of the serum of syphilitic patients or animals for virulent treponemes is described. In view of the significance of such a finding it is to be hoped that confirmation will be forthcoming.

Precipitins. Fornet and his collaborators (112) sought to demonstrate the presence of precipitins in the serum of patients with syphilis (particularly cases of paresis and tabes), and for this purpose used as antigen the serum of other syphilitics who possessed manifest lesions. They assumed that because such patients exhibited active manifest lesions their blood serum would be more apt to contain precipitinogen than the serum of syphilitics without obvious lesions, hence could be used as antigen. They found that when the serum of such patients was allowed to come in contact with the serum of other patients, whose infection was latent, a precipitate was formed in a large proportion of the cases. Normal serum did not give a precipitate under the same conditions. The formation of a precipitate under these circumstances was regarded by these investigators as an example of a true antigen-antibody reaction, and they thought it indicated the formation of specific precipitins in the body of the syphilitic individual. L. Michaelis (113) at the same time extracted the liver of a syphilitic fetus with salt solution and found that when this extract was brought in contact with the serum of a patient with hereditary syphilis a precipitate was formed. He expressed the thought that the reaction might ultimately supplant the Wassermann test as a diagnostic procedure, a rather prophetic remark in view of the apparently increasing popularity of precipitin tests as diagnostic

procedures in syphilis. Jacobsthal (114) later found that when the ordinary Wassermann "antigens" were brought in contact with serum from patients with syphilis a precipitate was formed which was visible under the microscope if dark field illumination were used. It was at first supposed that these precipitin reactions represented specific antigen-antibody reactions but this notion was soon abandoned. Despite this fact interest in the phenomenon was maintained and numerous attempts were made to modify the reaction so as to be able to utilize it for the diagnosis of syphilis. The names most prominently associated with this work are those of Bruck and Hidaka (115), Hecht (116), Meinicke (117), Sachs and Georgi (118), and Dreyer and Ward (119) abroad, and Kahn (120) in this country. Already a considerable literature on the subject has accumulated. The modification advocated by Kahn has been enthusiastically received in some quarters, where it has been substituted for the Wassermann reaction as a routine procedure in the diagnosis of syphilis. The test is, of course, an empirical one, and not a true antigen-antibody reaction in the strict sense of the term. Takenaka (121) has recently reported the occurrence of positive Sachs-Georgi reactions in the serum of syphilitic rabbits and finds that this test parallels the Wassermann test very closely in these animals. Specific precipitins for cultures of *Treponema pallidum* in the serum of patients with syphilis have not as yet been encountered although they were sought for by Arnheim (102). In Nakano's hands rabbits immunized with cultures of *T. pallidum* failed to develop precipitins in their blood for these organisms (89).

Complement-fixing substances. As is well known, the original complement-fixation reaction reported by Wassermann, Neisser and Bruck represented an attempt to demonstrate, in the serum of patients with syphilis, the presence of complement-binding substances for *Treponema pallidum*, presumably produced as a result of invasion of the body by the latter. In the absence of cultures of the organism resort was had to extracts of livers of syphilitic fetuses for antigens, since these organs were known to contain an abundance of treponemes. In spite of the heterogeneous mixture of chemical substances which such extracts must have contained, Wassermann's original conception was that the reaction represented a true antigen-antibody reaction. This idea had to be abandoned later when it was found that extracts of normal

organs would take the place of extracts of syphilitic fetal livers as "antigens" in the test, although for some years Wassermann clung to the idea that there was an element of strict antigen-antibody reaction in connection with the test.

As everyone knows, the literature that has accumulated in connection with the Wassermann reaction has become so enormous that it is almost beyond the capacity of any single individual to review it adequately, and this is scarcely the place to enter into a discussion of the various views that have been proposed to explain its nature. The concensus of opinion at the present time seems to be that it is a physical-chemical reaction connected in some way with the lipoids and syphilitic infection. Just why the serum of patients with syphilis and yaws should give this reaction and the serum of patients with other conditions should not give it, except in unusual instances, is not by any means clear. It should be mentioned at this point that recent reports from Germany suggest that there is an element of true antigen-antibody reaction in the Wassermann test (122). It is much too early to pass final judgment upon these most recent contributions to the nature of this intriguing test but it would be interesting indeed if the Wassermann reaction, after having been relegated to the limbo of empiric tests for so many years, should after all be haled back to the select company of strictly specific antigen-antibody reactions.

It may be noted in passing that the recent work of Takenaka (123), Wakerlin and Carroll (124), and Kemp, Chesney and Poole (125) indicates that with a prescribed technique syphilitic rabbits give positive Wassermann reactions during the period of manifest lesions and normal rabbits give negative reactions. This is not in accord with the traditional view prevalent in Germany, to the effect that the reaction cannot be relied upon in rabbits, but supports the earlier observations of Nichols (126).

Opsonins. So far as the author is aware, opsonins for *Treponema pallidum* have not been demonstrated in the serum of patients with syphilis. Nor has it been clearly shown that phagocytosis plays an important rôle in the mechanism of resistance to syphilitic infection. It is true that examples of phagocytosis of treponemes in syphilitic lesions have been described by Ehrmann (127) and Levaditi (128) but the experiments of Zinsser and Hopkins (129) with mice inoculated

intraperitoneally with *Treponema pallidum* would make it appear as if phagocytosis were of little or no importance in natural resistance to syphilitic infection. Bergel (130) has recently reported results which are in direct conflict with those of Zinsser and Hopkins. According to the former, phagocytosis of treponemes by lymphocytes and large mononuclear cells can be demonstrated to take place in the peritoneal cavity of rabbits, guinea pigs and mice, when these species are inoculated intraperitoneally with material containing treponemes. It is by no means easy to form a judgment as to the rôle played by phagocytosis in natural or acquired resistance to syphilitic infection because of the technical difficulties involved in the problem, and further studies along this line are urgently needed.

Cutaneous hypersensitiveness. Following the discovery of *Treponema pallidum* numerous attempts were made to extract from syphilitic tissue substances which, when injected into the skin of patients with syphilis, would provoke reactions of an allergic nature that would be specific and perhaps prove to be of diagnostic value. For the most part the livers of syphilitic fetuses constituted the source of this material. It is scarcely necessary to refer to the earlier work in great detail since the reports were very conflicting, as might indeed be expected in view of the varying chemical constitution of the mixtures obtained by extracting syphilitic tissue. While rather favorable results were reported by Meirowsky (131), Nicholas, Favre and Gauthier (132), and Nakano (133), negative or only slightly favorable results were obtained by Ciuffo (134), Jadassohn (135), Bertin and Le Bruyant (136) and Fontana (137).

The cultivation of *Treponema pallidum* in the test tube, however, offered greater prospect of success in this undertaking and led Noguchi to prepare an extract ("luetin") from cultures of this organism to be used as a skin test in the diagnosis of syphilis (138). Preliminary experiments with rabbits encouraged him to apply the test to human beings and several investigators beside himself reported favorable results obtained with the test, but the procedure has not made great headway as a means of diagnosing the presence of syphilitic infection, perhaps because of the fact that the Wassermann reaction has proved to be a more potent aid. The demonstration by Sherrick (139) that the administration of potassium iodide to patients with conditions

other than syphilis will frequently cause such patients to give a positive luetin reaction makes the test of limited value for diagnostic purposes. Nevertheless, Noguchi's work suggests that late in the course of syphilis there occurs a cutaneous hypersensitiveness to extracts of cultures of *Treponema pallidum*. Nakano (133) found that filtrates of cultures of this organism gave a higher percentage of positive skin reactions in syphilitic patients than did extracts of the same cultures, but that extracts of syphilitic livers gave the highest percentage of positive reactions. Nakano, furthermore, was able to show that the subcutaneous injection, in 2 human volunteers, of an extract of syphilitic liver, was capable of inducing in these individuals cutaneous hypersensitiveness to the same extracts injected intracutaneously. Similar results were obtained by this investigator in guinea pigs and rabbits. It may be noted in passing that Uhlenhuth and Mulzer were unable to obtain any evidence of the existence, in syphilitic rabbits, of cutaneous hypersensitiveness to syphilitic virus.

Attempts have not been lacking to demonstrate the presence, in the serum of syphilitic animals, of substances which, when transferred to guinea pigs, would passively sensitize these animals to material containing treponemes. Uhlenhuth and Mulzer injected guinea pigs intraperitoneally with 3 to 5 cc. of serum of syphilitic rabbits and inoculated them intravenously on the following day with approximately 1 cc. of an emulsion of syphilitic rabbits' testis. None of the guinea pigs showed anaphylactic symptoms. Nakano (133) apparently was able to sensitize guinea pigs by the intraperitoneal injection of extracts of syphilitic livers and subsequently to produce characteristic anaphylactic shock by the intravenous injection of the same extracts. Extracts of normal livers did not shock the animals. Evidence of the formation of anaphylatoxin in mixtures of syphilitic serum and extracts of syphilitic livers, in mixtures of cultures and guinea pig serum, and in mixtures of extracts of syphilitic tissue and guinea pig serum, was also obtained by this investigator.

Summary. It is clear from the foregoing analysis of the attempts to demonstrate the occurrence of specific antibodies in the course of syphilitic infection, that the subject is far from being settled. While one or another investigator has convinced himself that this or that kind of antibody can be shown to develop in patients with syphilis,

there are many conflicting reports. Nor is it by any means clear that the so-called antibodies that have been demonstrated have, in reality, anything to do with recovery from syphilitic infection or with acquired or natural resistance to the disease. It does seem definitely established that by the use of suitable procedures certain kinds of antibodies can be produced in the rabbit but when the very animal possessing these antibodies in its serum is found to be as susceptible to infection with potent virus as is a normal animal, one can scarcely escape the conclusion that such antibodies are of little value as a means of protection for the animal.

IMMUNOLOGICAL RELATIONSHIP OF SYPHILIS TO OTHER INFECTIONS DUE TO TREPONEMES

It may be of interest, before discussing the nature of immunity in syphilis, to consider the immunological relationship of this infection to those other infections (both of man and rabbits), which are caused by treponemes indistinguishable from *T. pallidum*. At the present time, as is well known, there is one such infection occurring in man (yaws), and one in rabbits (venereal spirochetosis), and it is generally conceded to be impossible, on morphological grounds, to differentiate the microörganisms causing these diseases from that causing syphilis.

Yaws

It is not within the province of this review to discuss in detail the question of the identity of yaws and syphilis. Considerable discussion has arisen in regard to it and there is as yet no unanimity of opinion although perhaps the majority of observers regard the conditions as separate disease entities. Leaving aside, then, the question of identity, is there any evidence that an attack of yaws is capable of protecting an individual against an attack of syphilis? According to Castellani and Chalmers (140) yaws patients are not immune to syphilis and vice versa. They cite the earlier observation of Charlouis (141) who inoculated a yaws patient with syphilitic virus and produced a characteristic attack of syphilis. At the time of inoculation this patient presented a yaws lesion and 4 weeks later there appeared at the site of inoculation (breast in this case) a typical indurated

chancre which was followed by axillary lymphadenitis and, later, a maculo-papular syphilide. As this experiment was carried out in 1880, long before the etiological agent of either of the diseases in question was known, the validity of the experiment rests upon the clinical diagnosis alone but the description of the lesions produced leaves little doubt as to the correctness of the diagnosis. In the same communication Charlouis described an instance which came under his observation where a patient with numerous yaws lesions contracted syphilis and the latter infection ran its usual course.

All of this evidence would tend to show that yaws confers no protection against syphilis, yet numerous observers have commented upon the fact that syphilis is not common in populations where yaws is widely prevalent. Parham (142) has made an interesting contribution to this problem. During a period of $2\frac{1}{2}$ years service in a Samoan hospital, which was the only institution of its kind serving a population of about 7000 natives, not a single case of syphilis was observed. Furthermore, an examination by this same investigator of the entire native population of one district, comprising about 3000 individuals, failed to reveal any cases of syphilis. Parham states that 100 per cent of the natives of Samoa acquire yaws before adult life and he raises the question as to whether the yaws infection may not protect against syphilis. He points out that the Samoans have been in intimate contact with whites since 1850 and that there would seem to have been adequate opportunity for syphilis to have been acquired by the former from the latter. Butler's experience in other islands of the Pacific has been similar to that of Parham and both are inclined to the view that in man yaws protects against syphilis (143).

The transmission of yaws to lower animals offered an opportunity to subject the problem to further study but the results obtained thus far have been somewhat conflicting. Neisser, Baermann and Halberstadter (144) found that a monkey (macaque) infected with syphilis was susceptible to infection with yaws. In their experiment, however, the inoculation with yaws was carried out 41 days after the inoculation with syphilis and only 15 days after the appearance of the primary syphilitic lesion. They found, also, that a monkey infected with yaws was susceptible to a subsequent inoculation with syphilitic virus, carried out 32 days after the inoculation with yaws but 14 days

before the primary yaws lesion made itself manifest. Levaditi and Nattan-Larier have criticized these experiments on the ground that the interval between inoculations was probably too short to have permitted an immunity to develop. While this criticism may be valid as applied to the experiments of Neisser and his coworkers, it is by no means certain that it is applicable to those of Halberstadter (145) and of Castellani (146). Both of these workers allowed a longer interval to elapse between inoculations and both found that yaws failed to protect monkeys against syphilis, and vice versa. On the other hand, Levaditi and Nattan-Larier themselves (147) found that when syphilitic monkeys were inoculated with yaws at intervals of 74 to 110 days after the inoculation with syphilis, they all proved refractory to the yaws infection. For the sake of convenience, the experiments upon monkeys have been assembled in tabular form (table 6).

Study of table 6 shows that in regard to the time interval between inoculations the experiments of Halberstadter and also Castellani correspond closely with those of Levaditi and Nattan-Larier, and yet the results are diametrically opposed. It would seem difficult to draw any final conclusions as to the immunological relationships between yaws and syphilis from the results thus far reported in monkeys. The problem has also been approached with the aid of rabbits. Recent experiments of Nichols, working with these animals, lends support to the contention that the time factor must be taken into consideration in evaluating cross protection experiments in syphilis and yaws. In his earlier experiments with rabbits Nichols (148) came to the conclusion that infection with yaws did not confer upon these animals protection against syphilis. In these experiments the interval between inoculations was comparatively short. Recently, however, he (149) has brought forward evidence to show that rabbits that have been infected with yaws and have carried their infection for a comparatively long period of time (91 to 376 days), whether treated or not, may in approximately 50 per cent of the cases be refractory to a subsequent inoculation with a strain of *T. pallidum* which is highly virulent for the rabbit. There is a close parallelism between the percentage of successful inoculations of syphilis obtained by Nichols in yaws rabbits, and the percentage of successful reinoculations ob-

TABLE 6

Cross inoculations of monkeys with yaws and syphilis

AUTHOR	ANIMAL	FIRST INOCULATION	INCUBATION PERIOD	INTERVAL BETWEEN INOCULATIONS	SECOND INOCULATION	RESULT	INCUBATION PERIOD
			days	*days*			*days*
Neisser, Baermann and Halberstadter	Mac. cyn.	Yaws	46	32	Syphilis	Positive	21
	Mac. nig.	Syphilis	26	41	Yaws	Positive	34
Halberstadter	Mac. cyn.	Yaws	49	73	Syphilis	Positive	22
	Mac. nem.	Syphilis	36	296	Yaws	Positive	46
	Mac. cyn.	Syphilis	52	58	Yaws	Positive	42
Castellani	Mac. pil.	Yaws	?	About 136	Syphilis	Positive	26
	Mac. cyn.	Yaws	32	101	Syphilis	Positive	42
Levaditi and Nattan-Larier	Mac. rhes.	Syphilis	15	74	Yaws	Negative	
	Mac. cyn.	Syphilis	20	91	Yaws	Negative	
	Mac. cyn.	Syphilis	22	91	Yaws	Negative	
	Mac. rhes.	Syphilis	19	95	Yaws	Negative	
	Bon chin.	Syphilis	28	110	Yaws	Negative	

tained by others in syphilitic rabbits when heterologous strains of *T. pallidum* were used for the second inoculation (see table 2). Voegtlin and Dyer (70) have also recently adduced evidence to show that treated syphilitic rabbits which are refractory to a second inoculation with syphilis are refractory to inoculation with yaws virus. Kolle (51) found that 7 syphilitic rabbits were all refractory to infection with yaws virus, while of 15 rabbits infected with yaws, 9, or 60 per cent, could be successfully infected with syphilis. In his experiments at least 120 days elapsed between inoculations.

Of interest is a recent experiment of Jahnel and Lange (150). These investigators inoculated 4 paretics with yaws virus which had been propagated in rabbits' testes. In none of the patients did a lesion develop although the virus was rubbed into scarified areas of the skin, injected intracutaneously and bits of infected rabbit testis were implanted in the deeper layers of the skin. That failure of the paretics to show lesions could not be attributed to avirulent virus was demonstrated by the fact that a normal individual inoculated as a control with the same material developed yaws lesions.

Summary. There is some evidence, of an epidemiologic nature, that in man infection with yaws protects against syphilis, although instances of both infections occurring simultaneously in the same individual have been reported. The evidence obtained from experiments with monkeys is somewhat conflicting, but recent work with rabbits suggests that there is a close biological relationship between the etiological agents of the two diseases. If it is true that an attack of yaws is capable of protecting man against syphilis one cannot help wondering if it might not be possible to protect human beings against syphilis by systematic inoculation with yaws virus, even to the extent of producing a mild attack of the latter. Perhaps the tropics will yet see a new Jenner. If there is anything in the idea that yaws protects against syphilis, one might be justified in raising the question as to whether campaigns to eradicate yaws are after all of real value in communities exposed to syphilis. Which would be better, yaws in childhood with concomitant immunity to syphilis later in life, or no yaws and susceptibility to syphilis?

Venereal spirochetosis of rabbits

This infection has of late attracted considerable attention because of the possibility of confusing it with experimental syphilis in the rabbit. It is an infection indigenous to rabbits, manifesting itself as a localized chronic dermatitis of the genitalia and anus. The lesions are superficial, scaly, and entirely without induration, and can scarcely be mistaken for syphilitic lesions. The disease appears to be more common in Europe than in America. It is transmissible from rabbit to rabbit by sexual intercourse, whereas *T. pallidum* infection in rabbits is not transmissible by that method.[7] The condition is universally held to be due to infection with a variety of treponeme (*T. cuniculi*) which is morphologically indistinguishable from *T. pallidum*. The experiments of Kolle, Ruppert and Möbus (151) demonstrate conclusively that there is no evidence of immunologic relationship between *T. pallidum* and this organism. Rabbits infected with the former are susceptible to infection with the latter and vice versa.

NATURE OF IMMUNITY IN SYPHILIS

The fact that a syphilitic individual, whether man, monkey or rabbit, acquires during the course of his disease a resistance of a rather high order to a second syphilitic infection, although it is impossible to confer this resistant state upon a representative of any of these species by any known method of immunization at the present time, has naturally led to much speculation as to the nature of this acquired resistance and the mechanism of its production. Obviously the circulating blood would be at once considered, particularly in view of the fact that in relapsing fever recovery can be shown to be accompanied by the appearance in the blood serum of spirocheticidal substances which are capable of exerting their action in the test tube. The proponents of the humoral theory of immunity in syphilis have, however, found but scant support amongst the known facts. There is really very little evidence, apart from the work of Eberson, to indicate that there are

[7] Marie, Levaditi and Banu (152) reported the transmission of their so-called "neurotropic" virus of syphilis from rabbit to rabbit by sexual intercourse but most workers in experimental syphilis are of the opinion that they confused *T. pallidum* with *T. cuniculi*, and that in reality it was venereal spirochetosis of rabbits that was transferred and not syphilis.

circulating antibodies in syphilis which play a rôle in the mechanism of acquired resistance to this infection. While there have been many references to the Wassermann reaction as indicating the presence of a circulating antibody in the blood, strictly speaking there is no evidence at present to show that the unknown substance or condition which gives rise to this reaction in the course of syphilis is at all concerned with the mechanism of recovery from or resistance to, this infection.

The general failure of investigators to demonstrate, in the blood serum of syphilitic patients or animals experimentally infected, the presence of substances antagonistic to virulent treponemes, has naturally directed attention away from the blood and toward the tissues as the seat of the resistance. There can be no question but that the tissues of the syphilitic are profoundly altered from those of the normal in their capacity to react to syphilitic virus introduced from without. They can be shown to be highly refractory to such exogenous virus and, in the great majority of instances, the virus is apparently completely disposed of without the development of gross signs of inflammation when it is introduced into the skin or subcutaneous tissue of syphilitic patients. Neisser was unwilling to apply the term "immunity" to this resistant state, however, for, as has been previously outlined, his own experimental work led him to conclude that it was dependent upon the persistence of foci of syphilitic infection in the body.[8] What others had regarded as true immunity Neisser contended was only an acquired inability of the skin to react to new virus introduced from without. According to him the condition might be regarded as merely one of acquired indifference to syphilitic virus, without the factor of protection for the host being necessarily implied. For this condition he proposed the term "anergy." He regarded the anergic state as relative and not absolute, and had the idea that it developed gradually and perhaps not at a uniform rate in all the tissues. He pointed out that it appears at about the time the Wassermann reaction becomes positive and he thought that it probably diminished in the later periods of the disease.

Kraus and Volk (22) proposed the view that the resistant state that

[8] This type of resistance was designated "Halbs-immunität" by Ehrlich. Recently Kolle has spoken of it as "Infektionsimmunität."

develops during the course of syphilis might be conferred upon certain tissue groups, the skin for example, and not upon others; in other words, that it was regional in distribution and perhaps not general. These workers found support for this conception of regional immunity in the immunological relationships that have been found to hold in the case of vaccinia. They called attention to the work of von Prowazek (153) and of Jurgens (154), which they were able to confirm, to the effect that inoculation of one cornea of a rabbit with vaccine virus did not protect the skin or the opposite cornea against subsequent inoculation with the same virus. They also showed that cutaneous inoculation of apes with vaccine virus and the production of a pustule did not protect the cornea against subsequent inoculation. These experiments offer strong support for the conception of a regional immunity in the case of vaccinia and present an attractive explanation by analogy for what has been observed in syphilis.

Subsequent reinoculation experiments by Tomasczewski with rabbits, to which allusion has already been made, may be regarded as offering support for this conception of regional tissue immunity in syphilis. It will be remembered that this investigator found that rabbits with pre-existing syphilitic orchitis could be successfully infected a second time by corneal inoculation, whereas the skin of such animals was refractory to the introduction of new virus. He found also that rabbits which had been inoculated in the cornea and in which keratitis had developed could be successfully inoculated in the testis. On the other hand, Uhlenhuth and Mulzer obtained directly opposite results.

Kraus was of the opinion that the skin participated in the immune process while the internal organs did not. This independent sharing by various tissues or organs in the immunizing process in syphilis finds support in the clinical observation that patients with tabes dorsalis or paresis rarely show syphilitic skin or bone lesions and rarely give a history of extensive eruptions. Neisser, however, was unable to admit that there was any evidence, from either the clinical or experimental standpoint, that a reciprocal relationship existed between immunity of the skin on the one hand and immunity of the internal organs on the other.

The conception that the resistant state which develops in individuals

during the course of syphilitic infection is not distributed uniformly throughout the body but that various tissue groups may share in it to an unequal extent under certain conditions has been strengthened by the work of Brown and Pearce (155). These investigators, working with rabbits, have come to the conclusion that the character of the initial response of the animal to syphilitic infection will determine the subsequent course of the disease so far as the evolution of generalized macroscopic lesions (detectible during life) is concerned; also that the occurrence of reactions in certain tissue groups tends to influence the occurrence of reactions in other tissue groups. The observations that they have been able to make have led them to formulate two laws which they speak of as the "law of inverse proportions" and the "law of progression or sequence." According to the former of these two laws the course of syphilis in the rabbit is in inverse ratio to the extent and intensity of the initial local reaction. In other words, in animals with marked outspoken primary reactions the subsequent course of the infection (number and extent of generalized lesions), is milder than in those animals in which the primary response is relatively slight or is suppressed by artificial means. Their second law, the law of progression or sequence, is based upon the observation that when syphilis is allowed to run its course undisturbed in the rabbit there tends to be an orderly development of metastatic lesions, those involving one tissue group tending to make their appearance before those involving other tissue groups. It is the conception of these investigators that this state of affairs reflects a "natural order of susceptibility and of involvement," and they consider that reactions taking place in one group of tissues may give rise to protective influences which are imparted to other groups of tissues, again in an orderly fashion. It is clear that this conception implies that the resistant state which develops during the course of syphilis is at least unequally shared by certain tissues, and it seems not unlikely that this inequality of participation in the immune reaction may explain the subsequent course of the disease in any given individual.

Largely upon the basis of his own work, Zinsser (4) has suggested that the refractory state which develops during the course of syphilitic infection may be the expression of a local immunity developing *in situ* wherever the microörganisms happen to lodge and set up a reac-

tion. Here then we have a narrower conception of syphilitic immunity than that of the regional idea advanced by Kraus. The evidence upon which Zinsser bases his proposal was derived from experiments by himself and co-workers upon a series of rabbits. These investigators found that "the opposite testis can be successfully inoculated before, during or after, the existence of a testicular lesion on on side, but that reinoculation of the same testis which had apparently returned to normal, at periods ranging from 6 weeks to one year, was not often successful." Study of the protocols of these experiments reveals the fact that in the instances where Zinsser and his collaborators obtained undoubted reinfections, heterologous strains of treponemes were used for reinoculation, whereas when homologous strains were used no reinfections were obtained. Because of the use, by these workers, of heterologous strains for reinoculation, and because of the now well extablished fact that in the rabbit the acquired resistance to syphilitic infection is more effective against homologous than against heterologous strains of treponemes, one is justified in being cautious before accepting the interpretation that Zinsser has put upon his experiments.

Chesney and Kemp (49) found that the opposite uninvolved testis of syphilitic rabbits treated late in the course of their infection was just as refractory to a second inoculation with homologous strains as a testis which had been the seat of a syphilitic inflammatory reaction prior to treatment. Moreover, in an experiment the report of which is now in course of publication these same investigators were able to show that granulating wounds in immune rabbits share in the anergic state to some, although not quite the same extent as do testes which were formerly diseased. In a series of 16 rabbits inoculated intratesticularly and treated late in the course of the disease, at a time when from previous experience one could be certain that they would be refractory to a second intratesticular inoculation, second inoculations were performed by applying the virus to a granulating wound (14 days old) on the back of the animal. In only one of the 16 animals did a lesion (chancre) develop at the site of inoculation; in the remaining 15 no lesion made its appearance but in at least 7 of these 15 there was evidence that infection had taken place and that dissemination of the virus had occurred. Since in this experiment the wounds were

made after thorough treatment and the granulation tissue represented new tissue which could scarcely have been exposed to invasion by treponemes until the time of the second inoculation, this experiment would tend to indicate that in order for tissue (of rabbits) to acquire the anergic state toward treponemes, it is not necessary that there be previous infection of that tissue by the organisms. Apparently in the rabbit, at least, the acquired refractory state or anergy or immunity, call it what you will, is a property which may be imparted to newly-formed granulation tissue but is not absolute, since under the conditions of the experiment systemic infection of some of the animals occurred even though no lesion developed at the site of inoculation.

This last fact, that syphilitic animals that have been treated can be successfully infected a second time without the development of any lesion at the site of reinoculation, has been substantiated in several experiments performed by the writer in association with Kemp, and it suggests that in immune animals the circulating blood does not share in the resistant state to the same extent as the tissues at the site of reinoculation. If, as these investigators have demonstrated, the resistant state can be conferred upon newly-formed granulation tissue and if, in addition, it can be shown that there is no humoral factor operating in syphilitic immunity, one may perhaps conclude that the resistant state is confined to the cells and can be transmitted to successive generations of new cells. On the whole, there is more evidence in favor of the view that the mechanism of immunity in syphilis is more closely related to the cells than to the blood, although neither of these factors can be ruled out at the present time and it is not unlikely that both are involved.

It might be supposed that phagocytosis would play a rôle in the mechanism of acquired immunity in syphilis but thus far little evidence has been forthcoming to indicate that it is of any significance, either in natural or acquired resistance to this infection. Little is known of the extent to which it enters into the process of healing of the initial manifestations of the disease. While both Ehrmann and Levaditi claim to have observed in stained sections examples of what they regarded as phagocytosis of treponemes, and Bergel attaches great importance to the process, the experiments of Zinsser and Hopkins on mice, on the other hand, make it seem likely that it is not of

major importance as a defensive mechanism for animals not inherently susceptible to syphilitic infection. Moreover, the ease with which granulating wounds of normal rabbits can be inoculated with virulent strains of *T. pallidum*, as demonstrated by Chesney and Kemp (156), does not lend support to the idea that the phagocytic cells of the rabbit are particularly active against living virulent treponemes. Nevertheless, the reservation should be made that in the immune animal phagocytosis may have a much more important part in the defensive mechanism than can be determined at the present time with the methods at our disposal.

This phase of the subject should not be dismissed without calling attention once more to the observations of Bergel (130). This investigator has come to the conclusion that the lymphocytes and macrophages are of prime importance in the destruction of treponemes in the body of the host. He is convinced that phagocytosis of these organisms by these particular cells takes place with great frequency in syphilitic infections, and he claims to have observed in fresh specimens of material from syphilitic lesions the engulfing of living, actively motile specimens of *T. pallidum* by lymphocytes and macrophages and their subsequent dissolution within the cell. He is of the opinion that both extra- and intracellular lysis of treponemes is brought about through the agency of lipolytic enzymes which the lymphocytes are capable of elaborating and discharging into the surrounding medium. His conception of the defensive mechanism in syphilis, then, is that it is in large part a reaction taking place between the lipoidal treponemes on the one hand, and lipolytic enzymes evolved by the lymphocytes on the other, with destruction of the former. It is clear from a perusal of Bergel's communication that much of his hypothesis rests upon the interpretation of stained preparations checked by examination of fresh preparations observed by the dark field method. Because of the dangers of misinterpretation of stained specimens, opinion as to the views of Bergel must be guarded for the time being; nevertheless, the conception is new and offers the possibility of a fresh line of attack.

If it is an established fact that human beings with syphilis acquire, early in the course of their disease, a resistance to new infection from without, to the extent that the skin is wholly refractory to relatively large amounts of foreign virus introduced upon a scarified area, how

can one reconcile with this state of affairs the fact that such individuals may, and not infrequently do, continue to show lesions on their skin, mucous membranes and elsewhere, caused by their own virus coming from within? Here apparently is a paradox—acquired resistance to exogenous virus but continuing susceptibility to endogenous virus. This peculiar situation has for years been a matter of keen interest to students of syphilis. As long ago as 1884 Neisser (157) called attention to it and in the intervening years several hypotheses have been offered in explanation.

It has been suggested that the superficial layers of the skin acquire the refractory state in advance of the deeper layers so that the host is protected against outside virus before he is protected against that coming from within. Against this view is the well known fact that secondary lesions of syphilis do eventually involve the most superficial layers of the integument. Another view that has been proposed is that from time to time there occur fluctuations in the refractory state which permit successive crops of treponemes to be engendered, these in turn producing lesions. It must be admitted that the existence of such fluctuations has not been proved and it seems unlikely that they occur during the early stages of the disease at any rate. It is conceivable that the apparent paradox might be explained upon the basis of Zinsser's theory, namely that the resistant state is a local phenomenon dependent upon direct invasion of the tissues by the parasites. According to this conception if lesions developed subsequently in a given tissue or organ it would be because that particular locality had not been invaded at the time of the first dissemination of the treponemes or at any rate had not been the seat of an inflammatory reaction. Still another hypothesis that might explain the observed facts would be the supposition that *Treponema pallidum* possesses a complicated life cycle during the course of which it enters phases which are more virulent or more resistant against the body defenses. Nothing definite is known of a life cycle for this organism in which the parasite takes on a new or different morphology from that with which we are now familiar, although at least one author, McDonagh, (158) has been insistent in his claims that there is such a complex cycle, and not a few others have toyed with the idea.

The hypothesis to which both Neisser and Levaditi lend their adher-

ence, in order to explain the apparent paradox, holds that the treponemes develop a resistance against the treponemicidal agents of the host, that the former themselves become immunized as it were and in this manner are able to resist a defensive mechanism which is fully capable of dealing with and destroying foreign virus. One may conceive of a balance being struck between the host and the parasite, a mutual immunization as it were, whereby the latter remains viable for decades in the body of the former without exerting any tissue reaction and perhaps without increasing in numbers, yet is ever ready and able to initiate an inflammatory reaction should conditions (as yet imperfectly understood) favor the development of new generations of organisms, and retains, as a parasite, the ability to infect and produce disease in a new host. This state of affairs implies a delicate adjustment between host and parasite; actually, of course, it is but another way of describing the course of syphilitic infection as observed in man. As Levaditi has pointed out, it presupposes a certain plasticity on the part of the treponemes, but there is no reason to suppose that such a condition might not be inherent in these organisms, or possibly be developed in them in response to the proper demand. Indeed the experiments of Zinsser and his co-workers make it seem entirely likely that treponemes do possess plasticity in the sense that they are capable of undergoing profound changes in both virulence and susceptibility to antagonistic agents. Attention has already been called to the experiments in which these investigators demonstrated that when *T. pallidum* was cultivated in the test tube the microörganisms lost all virulence for rabbits and were readily agglutinated and underwent lysis in the serum of immunized animals whereas the same strain, while being propagated in rabbits, not only retained its virulence but was incapable of being agglutinated or dissolved by the same serum.

The hypothesis that an equilibrium is set up between host and parasite through a process of mutual immunization or adaptation, attractive as it may be, still leaves unexplained the causes for the disturbances in this equilibrium which later bring about manifestations of disease. One must assume, in accordance with this theory, that such disturbances do occur in order to account for the subsequent development of manifestations of syphilis after a period of latency. Eberson's view is that treponemicidal activity evolved by the host in response to

invasion by the treponemes is responsible for the maintenance of the latent state, but he gives no explanation for the subsidence of this activity and the attendant resumption of the disease process.

When for reasons as yet obscure the balance is disturbed and new inflammatory reactions are set up with the development of macroscopic lesions, do these new lesions represent an increase in the number of treponemes or an exaltation of their virulence, or both, and is the reaction similar to that which would be exhibited by a normal individual or one in the early stages of the disease? Or is there evidence that the body of the syphilitic individual, by reason of the existence of syphilitic infection within it for some time, has been changed and now reacts in an altogether different manner? All the evidence is in favor of the latter view. Clinical and pathological experience, as is well known, shows that the tissues of the syphilitic individual, who has carried his infection for some time, react toward his own virus in a manner altogether different from that of a normal person. The character of the late lesions is markedly different from that of the early ones—destructive and proliferative changes are in the ascendency in the case of the former, and yet it is the universal experience that treponemes are extremely scarce in tertiary lesions. The inflammatory response in late syphilis is indeed out of proportion, apparently, to the size of the stimulus, and this indication is sufficient to place the late tertiary lesions in the category of allergic phenomena. Moreover, animal inoculation has demonstrated that the organisms found in these lesions do not possess an exalted virulence, hence there is no reason to ascribe to changes in the virulence of the virus, the greater tissue response exhibited by the late lesion. Rather, on the other hand, should it be ascribed to changes in the host.[9]

[9] Bergel (130) has suggested an explanation for the difference in character between early and late lesions which emphasizes changes in the parasite rather than in the host. His view is that in the course of syphilitic infection the inciting agent is altered by reason of the defensive mechanism of the host, that this alteration is expressed in a change in the chemical constitution of the virus, and that the chemically altered virus is capable of setting up a different sort of cellular reaction from that occasioned by the unaltered virus. He thinks that the cellular picture of an inflammatory reaction is conditioned in part by the chemical nature of the antigen, that in early syphilis the reaction is primarily lymphocytic, due to the lipoid nature of the (as yet) unaltered treponemes, but that later, when through the action of lipolytic enzymes the treponemes are divested of their lipoidal constituents, the chemical constitution of these organisms is changed and con-

It appears, then, that there develops in the syphilitic individual in the course of time, a state characterized by an alteration in the way in which his tissues react to his own or foreign virus. This alteration may take the form either of over-activity, or of indifference toward the virus. The changed reaction capacity, as manifested by over-activity, has been called by Neisser "Umstimmung," and is synonymous with allergy. To that altered reaction capacity of the syphilitic which manifests itself as indifference to virus he has given the term "anergy." It is his idea that the anergic and allergic states are not fixed states in the body of the syphilitic individual but that they are continually undergoing alteration, probably tending to disappear or at any rate diminish in intensity as time goes on. It is by no means clear that they are to be regarded as two independent processes; perhaps one is the natural outcome or development of the other. They may very well represent phases or functions of the immunizing process, that is, parts of the protective mechanism, and it is possible to conceive of explaining the entire course of syphilis upon the basis of alterations in these states, affecting in turn the activity of the treponemes.

One often sees the statement that immunity in syphilis is different from that seen in other infections. Proponents of this view rest their case largely upon the contention of Neisser that there is no true immunity in syphilis, but merely a resistance to new infection so long as the first infection is present. They would almost infer an entirely different sort of defense mechanism for syphilis as opposed to other infections, a mechanism that is always in need of stimulus in order to function and that ceases to function as soon as the stimulus has been removed. Rather, it seems to the writer, the emphasis should be placed upon the difference in the reaction to the infection during the early period of the disease. In syphilis we have an example of a slowly developing, incomplete or partial reaction to the invading organism, neither party getting the upper hand in a short space of time and overwhelming the other, as happens in pneumococcus infections, for example. It is as if time were given for the development of a mutual adjustment between host and parasite, enabling them to live side by

comitantly their antigenic power is altered so that instead of evoking a reaction which is predominantly exudative, they now induce a granulomatous reaction which may lead to necrosis or to connective tissue proliferation.

side for years, and affording an exquisite example of parasitism. That the host reacts against the invader cannot be denied, that in the long run the former plays the dominant rôle in the transaction is also true, even if the invader may ultimately bring about the death of the host, but the essential disease picture of syphilis is that of a prolonged truce between two contenders, if we may use the military metaphor, interrupted from time to time by renewed encounters it is true, but on the whole a long drawn out struggle in which neither antagonist is quite able to overcome the other.

That a high degree of resistance to syphilitic infection develops in the body of him who acquires the disease cannot be doubted, but that this resistance ever attains the point where there is complete sterilization of the body is extremely unlikely. It would appear from our present knowledge that the most we can expect the unaided human body to do when confronted with this particular infection is to acquire a resistance that will serve to keep the infectious agent in check for long periods of time. It by no means follows, however, that it is necessary for the infectious agent to remain in the body in order that this resistance may be maintained. It is upon just this point that the author is inclined to disagree with most of his predecessors in this field, although admitting that the question is still an open one. If acquired immunity in syphilis is dependent upon the persistence of active foci of syphilitic infection somewhere in the body then it would be reasonable to argue that the immunizing process in syphilis is quite different from that observed in most other infections; however, the truth of the former assertion is regarded by the author as not yet definitely established and until it is, there is no necessity to assume that in syphilis we have to do with a defensive mechanism which is fundamentally different from that which operates in other infectious diseases. It may be quantitatively different, but not necessarily qualitatively different.

One may speculate as to the failure of the body completely to react against the syphilitic virus and bring about its destruction. Is it because the organism can wall itself off from attack? Or is it not perhaps due to a fundamental similarity between the life processes of the human cell on the one hand and the treponeme on the other? It is conceivable that there may be just enough difference between the

treponemes and the body cells to evoke an inflammatory reaction but not enough to make this reaction overwhelming for the former. In the final analysis the difference or the similarity must be a metabolic one and perhaps we shall have to wait for a clearer understanding of the physiological processes of the treponemes and body cells as well, before light is thrown on the question. To make my meaning clear in this connection, I cannot do better than quote from Theobald Smith (159) who wrote in 1913 as follows: "There is another type of parasite which may dispense largely with both offensive and defensive processes. We can conceive of this type as exerting a metabolic activity approximating so closely to that of the host that the latter reacts but slightly, and then only after a long period of stimulation. This type of parasite would have very little that is body-foreign or blood-foreign. In this latter class I would place such organisms as the spirochete of syphilis. If the exquisite, prolonged parasitism of this microörganism, the relatively slight lesions and their curability, be borne in mind, it will be understood why I place it in this category."

GENERAL SUMMARY

Thus far the only animal species that have been found to be regularly susceptible to infection with *T. pallidum* are man, the monkey and the rabbit.[10] There is no evidence to show that any races of man are naturally insusceptible to syphilis, although some races do seem to react differently from others to the infection. Of natural individual immunity to syphilis nothing is known.

With reference to acquired immunity to syphilis, clinical experience has demonstrated that second attacks of this infection in the same individual are extremely rare. Inoculations of patients in various stages of the disease with active syphilitic virus have shown that the syphilitic individual gradually acquires a resistance against luetic virus introduced into the skin from without. This resistance is not absolute, however, but appears to be more pronounced during the later stages of the disease. Inoculation of patients presenting secondary or tertiary manifestations of syphilis with virulent syphilitic virus

[10] Since the above was written Kolle and Evers (163) have reported the successful transmission of syphilis to guinea pigs with such regularity, apparently, as to permit the utilization of this species for the experimental study of syphilis.

under certain circumstances leads to the development of lesions simulating those characteristic of the particular stage of syphilis in which the individual happens to be. Such inoculations very rarely lead to the production of a chancre followed by secondary manifestations, such as occurs in the ordinary evolution of the disease in a normal individual.

Experiments show that syphilitic monkeys and rabbits also acquire a resistance to a second infection. In the rabbit this resistance is first manifest about 6 to 8 weeks after inoculation and appears to be firmly established by the fifteenth week of the disease. In the monkey, also, the resistance requires time for its development. There is some experimental evidence which indicates that in the rabbit the refractory state is not equally imparted to all tissues of the body.

Neisser advanced the view that the acquired refractory state in syphilis is dependent upon the persistence of foci of syphilitic infection. Recent experimental evidence suggests that rabbits, at least, may acquire during the course of syphilis an immunity which will persist after the infection has been abolished. Treatment of syphilitic rabbits at appropriate intervals after infection is capable of arresting the development of this resistant state but not of abolishing it, once it is established. Clinical experience in respect of the occurrence of reinfections in man is not in conflict with the conception of an acquired immunity which persists in the absence of the disease. This conception, if fully substantiated, will dispose of the reinoculation test as a criterion of cure of syphilis.

Recent experiments with rabbits show that the acquired refractory state which develops in these animals during the course of syphilitic infection is more effective against a second inoculation with homologous strains than with heterologous strains. There is also evidence to show that it is possible to reinfect treated syphilitic rabbits without the development of a characteristic syphilitic lesion at the site of reinoculation. The time at which treatment is begun and the mode of reinoculation appear to be two factors which play a part in bringing about this type of reaction.

Attempts to produce in man or the lower animals, active immunity to syphilitic infection by the utilization of derivatives of syphilitic tissue, cultures of treponemes, syphilitic virus (living or dead), or

products of the latter, have been uniformly unsuccessful, provided the disease itself was not produced. Nor have such methods had much effect upon the course of an already established infection. Attempts to confer immunity to syphilis by passive transfer of serum from immune persons or animals have likewise been fruitless. Apparently infection with virulent virus is necessary to produce immunity, although perhaps not essential to maintan it.

It has been possible to demonstrate antibodies such as agglutinins, precipitins and treponemicidal substances in the serum of animals following immunization with virus from syphilitic tissue, or more particularly with cultures, and some observers have reported the presence of these antibodies in the serum of patients with syphilis, but it is not at all clear that these substances have anything to do with natural or acquired immunity to this infection. It is significant that they have been found to be less effective against virus obtained from syphilitic lesions than against cultures of the organism.

There seems to be a biologic relationship between syphilis and yaws, although this question is by no means settled. There is as yet no evidence of any biologic relationship between *T. pallidum* and the organism causing venereal spirochetosis (*T. cuniculi*) of rabbits.

The mechanism of immunity in syphilis is not by any means clearly understood. There is on the whole little evidence that antibodies circulating in the blood are responsible for acquired resistance. The part played by the cells is not fully established although these are undoubtedly of prime importance in the resistance mechanism. It is certain that the tissues of the syphilitic individual acquire the capacity to react to new syphilitic virus introduced from without and also to the individual's own virus, in a manner wholly different from that manifested by the normal individual. This altered reaction capacity may at times manifest itself as a state of apparent indifference or anergy, again as a state of hypersusceptibility or allergy. Both of these states may be functions of the defensive process and may serve a useful purpose so far as the individual is concerned. Spontaneous sterilizing immunity in syphilis, if it ever occurs, must be extremely rare. Man seems to be incapable, as a rule, of eliminating syphilitic infection from his body unaided, although he does possess the ability to react against it and to limit somewhat its extent. This reaction

at its best is incomplete, however, although fairly effective for long periods of time. The fact that it is incomplete may be due in part to an immunization of the invading organism against the host, so that a balance is struck between the two, a mutual adjustment as it were, whereby a state of ideal parasitism is approximated, or else to some similarity between the metabolic activities of treponemes on the one hand, and the body cells on the other.

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